EVALUATION OF ANTI NEPHROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *APIUM GRAVEOLENS SEEDS* ON ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED UROLITHIASIS IN MALE WISTAR ALBINO RATS

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THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

in

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by

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Register No: 261525012

Under the Guidance of

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DECLARATION

Register No. 261525012, hereby declare that this dissertation entitled, "**EVALUATION OF ANTI NEPHROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *APIUM GRAVEOLENS SEEDS* ON ETHYLENE GLYCOL AND AMMONIUM CHLORIDE INDUCED UROLITHIASIS IN MALE WISTAR ALBINO RATS"** has been originally carried out by me under the guidance and supervision of **Prof. Dr.P.Amudha, M.Pharm,. PhD,** Asst professor for the department of pharmacology, C.L. BaidMetha College of Pharmacy, Chennai-97 for the academic year 2016-2017. This work has not been submitted in any other degree at any other university.

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I am making this project not only for marks but to also increase myknowledge.

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Sumalatha S

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LIST OF ABBREVIATIONS

µl	Micro litre
AG	ApiumGraveolens
ALT	Alanine amino Transferase
ANSA	8-Anilino -1-Naphthalene Sulfonic acid Ammonium salt
BBC	British Broadcasting Corporation
CAM	Complementary And Alternative Medicine
COM	Calcium Oxalate Monohydrate Crystals
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments
CaOx	Calcium Oxalate
CT	Computed Tomography
DNA	Deoxy ribonucleic Acid
EG	Ethylene Glycol
EHL	Electrohydraulic Lithotripsy
ESWL	Extra Corporeal Wave Lithotripsy
GSH	Glutathione S-transferase
HCL	Hydrochloric acid
EEAG	Ethanolic extract of Apiumgraveoelens
IAEC	Institutional Animal Ethics Committee
Kg	Kilogram
Mg	Milligram
NGF	Nerve Growth Factor
NHANES	National Health and Nutrition Examination Survey
PCN	Percutaneous Nephrolithotomy
PO	Post oral
SEM	Standard error mean
SGOT	Serum Glutamic Oxalo-acetic transaminase
SGPT	Serum Glutamic Pyruvic transaminase
SWL	Shock Wave Lithotripsy
TNF	Tumor Necrosis Factor
UK	United Kingdom
UTI	Urinary Tract Infections
WHO	World Health Organisation

LIST OF FIGURES

DEDICATED TO MY PARENTS,
MY PROFESSOR'S AND
MY FRIENDS

1. INTRODUCTION

1.1.Introduction to Herbal medicine

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundred of years, before the development and spread of modern medicine are still in use today .Or say , traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. The traditional preparations comprise medicinal plants, minerals ,organic matters for medicinal purposes, and the investigation of such use. Plants were been the basis for medical therapies through much of human history, and such traditional medicineis still widely practiced till date[1]. Modern medicine distinguishes herbalism as a form of alternative medicine, as the practice of herbalism is not strictly based on evidencegathered using the scientificmethod.

Archaeological proof indicates that the use of medicinal plants dates at least to the Paleolithic, approximately 60,000 years ago. Written evidence of herbal medicines dates back over 5,000 years, to the Sumerians, who created lists of medicinal plants. A number of ancient cultures wrote on plants and their medical uses. In ancient Egypt, herbs are cited in Egyptian medical papyri, portrayed in tomb illustrations, or on rare occasionsfoundinmedicaljarscontainingtraceamountsofherbs. Theearliest recognized Greekherbals were those of Diocles of Carystus, written during the 3rd century B.C, and one by Krateuas from the 1st century B.C. Only a few fragments of these works have lasted intact, but from what remains scholars have noted that there is a large amount of overlap with the Egyptian herbals[2]. Herbs were also common in the medicine of ancient India, where the prominent therapy for diseases was diet. De MateriaMedica by Pedanius Dioscorides a Roman physician, is a particularly important exampleof such writings. The certificationof herbs and their uses was a central part of both Western and Eastern medical scholarship through to the 1600s, and these works played amajor role in the development of the science of botany.

1.2.Prevalence of use

A survey published in May 2004 by the National Center for Complementary andAlternative Medicine focused on who used complementary and alternativemedicines (CAM), what was used, and why it was used. The survey was limited to adults, aged 18 years and over during 2002, living in the United States. According to the survey, herbal therapy, or use of natural products other than vitamins and minerals, was the most regularly used CAM therapy (18.9%) when all use of prayer was excluded[3].

Herbal remedies are very common in Europe. In Germany, herbal medications are dispensed by apothecaries (e.g., Apotheke). Prescription drugs are sold alongside essential oils, herbal extracts, or herbal teas. Herbal remedies are seen by some as a treatment to be preferred to pure medical compounds which have been industrially produced

.

1.3.Traditional herbal medicine system

Native Americans medically used about 2,500 of the approximately 20,000 plant species that are native to North America.

Some researchers skilled in both western and traditional Chinese remedieshave attempted to deconstruct ancient medical texts in the light of modern science. This interpretation is supported by several investigations of the ORAC ratings of various yin and yang herbs.

In India, Ayurvedic medicine has quite complex formulas with 30 or more constituents, including a sizable number of ingredients that have undergone "alchemical processing", chosen to balance "Vata", "Pitta" or "Kapha".

In Tamil Nadu, people have their own medicinal system now popularly called Siddhamedicine. The Siddha system is entirely in the Tamil language. It contains roughly 300,000 verses covering various aspects of medicine. This work includes herbal, mineral and metallic compositions used as medicine. Ayurveda is in Sanskrit, but Sanskrit was not generally used as a mother tongue and hence its medicines are mostly taken from Siddha and other local traditions.

1.4.Modern herbal medicine

Many of the pharmaceuticals currently accessible to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. According to theWorld Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. At least 7,000 medical compounds in the modern pharmacopoeia are obtained from plants among the 120 active compounds currently derived from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional usage of the plants from which they are derived[4]

1.5.Herbal preparations& Safety of Herbal Medicines

There are many forms in which herbs can be administered, the most common of which is in the form of a liquid that is drunk by the patient either an herbal tea or a (possibly diluted) plant extract. Whole herb consumption is also practiced either fresh, in dried form or as fresh juice.

Several standardization methods may be determining the amount of herbs used. One is the ratio of raw materials to solvent. However different specimens of even the same plant species may vary in chemical content. For this reason, thin layer chromatography is sometimes used by growers to assess the content of their products before use. Another method is standardization on a signal chemical[5].

Safety of Herbal Medicines

A number of herbs are believed to be likely to cause adverse effects. Additionally "adulteration, unsuitable formulation, or lack of study on plant and drug interactions have caused to adverse reactions that are sometimes life threatening or lethal. Proper double-blind clinical trials are needed to decide the safety and efficacy of each plant before they can be recommended for medical use. Although many consumers trust that herbal medicines are safe because they are "natural", herbal medicines and synthetic drugs may interact, causing toxicity to the patient.

Standardization of purity and dosage is not authorized in the United States, but even products made to the same specification may differ as a result of biochemical variations within a species of plant. Plants have chemical defense mechanisms against predatorsthat can have adverse or lethal effects on humans.

Examples of extremely toxic herbs include poison hemlock and nightshade. They are not

marketed to the public as herbs, because the risks are well recognized, partly due to a long and colorful history in Europe, associated with "sorcery", "magic" and intrigue. Although not common, adverse reactions have been reported for herbs in extensive usage. On circumstance serious untoward outcomes have been related to herb ingestion. A case of major potassium depletion has been attributed to chronic licorice ingestion. And consequently professional herbalists avoid the use of licorice where they recognize that this may be a risk. Black cohosh has been concerned in a case of liver failure. Few studies are available on the safety of herbs for pregnant women and one study found that use of complementary and alternative remedies are associated with a 30% lower ongoing pregnancy and live birth rate during fertility treatment. Examples of herbal therapies with likely cause-effect relationships with adverse events include aconite, which is often a legally restricted herb, ayurvedic medicines, broom, chaparral, Chinese herb mixtures, comfrey, herbscomprising certain flavonoids, germander, guar gum, liquorice root, and pennyroyal.[6]

2. LITERATURE REVIEW

2.1.URINARY SYSTEM INTRODUCTION

The Urinary System is a group of organs in the body filtering out excess fluid and other substances from the bloodstream. The elements are filtered out from the body through urination.. Urine is produced by the kidneys, accumulatedin the bladder and defecated through the urethra. Urine is used to remove excess minerals or vitamins as well as blood corpuscles from the body. The Urinary system functions with the other systems of the body to assist maintaining homeostasis. The kidneys are the main organs of homeostasis because they keep the acid base balance and the water salt balance of the blood.

2.2.UROLITHIASIS - INTRODUCTION

Stones that are formed within the urinary tract are known as urolithiasis or calculi. When crystals are found in urine, oversaturation with the chemical components has materialized, but this may have been in vitro, due to temperature or pH variations, so the existence of crystalluria alone does not endorse a diagnosis of urolithiasis. Diagnosis can only be confirm urolithiasis being identified in freshly voided urine, on radiography, surgery,or atultrasound. Radiolucent uroliths and radiodense uroliths less than two millimetres in diameter may be difficult to identify on plain radiographs, but the use of double contrast methods greatly increases the detection rate. Uroliths may form in the upper or lower urinary

tract, although clinical signs are usually related with lower urinary tract disease. Many animals with radiographic indication of struvite uroliths in the urinary bladder have nephroliths as well.

2.3.EPIDEMOLOGY OF UROLITHIASIS

The epidemiology of urolithiasis varies according to geographical area in term of prevalence and age,incidence, and sex distribution, stone composition and stone location. Such differences have been explained in terms of race, diet and climate factors. Socio-economic conditions have generated difference in the prevalence, incidence and distribution for age, sex and type of lithiasis in terms of both the site and the chemical-physical composition of the calculi.

Epidemiological surveys proves that the prevalence rate ranged between 4% and 20% for economically developed countries.

Urolithiasis, urinary stone development, is the third most common problem of the urinary tract, with lifetime incidence of 12% and 7% in men and women respectively in U.S.A. and 34% and 6-9% in women and men respectively in the other western countries[7,8]. In Indiaits reversion rate is about 50% in 5-10 years and 75% in 20 years[9,10]. The disease lead to loss of about $5 billion per annum in the USA [5]. About 12% of India population are suffering from the problem of urinary stones, 50% of which may result in kidney and renal injury[11]. About 80% of these calculi are made up of calcium oxalate (CaOx).

In the 20th century,occurrence and frequency of upper urinary tract stones were still increasing in Western countries probably resulting from developments in clinical-diagnostic procedures and differences in nutritional and environmental factors. Endemic infantile bladder stone disease, with features similar to those previously defined in Europe in the 19th century, was fairly prevalent in huge areas of Turkey, Iran, India, China, Indochina and Indonesia with stones composed of calcium oxalate and ammonium urate due to malnutrition.

2.4.ETIOLOGY OF UROLITHIASIS

Urolithiasis is a heterogeneous disorder, with changing chemical composition and pathophysiologic background. Although kidney stones are commonly composed of calcium phosphate or calcium oxalate, they may also consist of magnesium-ammonium phosphate, uric acid, or cystine. Stones mature from a wide variety of metabolic or environmental disturbances, including varying forms of undue hyperoxaluria, urinary acidity, hypocitraturia,

hypercalciuria,hyperuricosuria, infection with cystinuriaand urease-producing organisms. The cause of stone formation may be determined in most patients using the reliable diagnostic protocols that are available for the recognition of these abnormalities. Active medical treatments, capable of rectifyingessential derangements, have been framed. They comprise sodium cellulose phosphate, thiazide, and orthophosphate for hypercalciuric nephrolithiasis; acetohydroxamic acid for infection stones; potassium citrate for hypocitraturic calcium nephrolithiasis and d-penicillamine and α-mercaptopropionylglycine for cystinuria. Using these treatments, new stone development can now be prohibited in most patients[12].

2.5.TYPES OF KIDNEY STONES

There are four different types of kidney stones.

Figure no. 1

Calcium stones

Most kidney stones are composed of calcium particularly calcium oxalate. Calcium phosphate and other minerals also may be present. Conditions like hyperparathyroidism, increase the chance of calcium stones. Increased levels of oxalate also improvesthe risk for calcium stones. The calcium stone was shown in **Figure no. 1**.Certain medicines may prohibit calcium stones.

Uric acid stones

Figure no. 2

Some kidney stones are made of uric acid, a waste byproduct generally passed out from the body via urine. You are more probable to have uric acid stones if you have:

- Low urine output.
- A diet high in animal protein (red meat).
- An increase alcohol consumption.
- Gout.
- Inflammatory bowel disease.

The uric acid stone was illustrated in **Figure no. 2**

Certain medicines may inhibit or dissolve uric acid stones.

Figure no. 3

Struvite stones

Some kidney stones are struvite stones. They can also be termed as infection stones if they occur with kidney or urinary tract infections (UTIs).

Struvite stonesof kidney stones at times are also called staghorn calculi if they developbig enough.Struvite stones can be severe, because they are regularly large stones and may show up with an infection. Medical therapies, including antibiotics and elimination of the stone, is usually required for struvite stones. Women are more favorites than men because of their higher risk of urinary tract infections. These stones were illustrated in **Figure no. 3**.

Cystine stones

Figure no. 4

Less common are kidney stones made of a chemical called cystine. Cystine stones (illustrated in **Figure no. 4**) are more likely tohappenpeople whose families have a condition that results in too much cystine in the urine (cystinuria).

Cystine stones may be dissolved or prevented with medicine. But this may be difficult and not very effective. If a stone causes obstruction in the urinary tract or is too large, then it willneed to be removed[13].

2.6.CAUSES OF KIDNEY STONES

The important cause of kidney stones is a deficiencyof water in the body. Stones are more normally found in individuals who consume less than the suggested eight to ten glasses of water a day[14].

When there is not sufficient water to dilute the uric acid (a component of urine), the urine becomes more acidic. An extremely acidic environment in urine is favorable to the formation of renal stones. Medical conditions such as urinary tract infections, Crohn's disease, renal tubular acidosis, medullary sponge kidney, hyperparathyroidism,and Dent's

disease increase the threat of kidney stones.

Causes of Calcium stones

Kidney stones are common, affecting as many as 15 percent of people. These stones come in different varieties, with the majority of them including a calcium component. Calcium oxalate stones are the most common type and represent 56 to 80 percent of cases in adults. Understanding what causes these types of kidney stones can help you prevent them.

Kidney Stones Form Crystals in the urine all the time. They are usually small and passed painlessly. Kidney stones form when conditions allow these crystals to grow in size. In particular, excessive amounts of calcium, oxalate, phosphate, uric acid or cystine in the urine can lead to kidney stones. Substances exist normally in urine that can prevent kidney stones from developing. Magnesium, citrate, pyrophosphate and other enzymes all act in the body as a deterrent to crystals forming and attaching to the surface of kidney tubes. Having too little of these substances present in urine can trigger kidney stones[15].

2.7.FACTOR'S INDUCING LITH'S FORMATION

High levels of calcium

Too much calcium in the urine -- hypercalciuria -- can be a risk factor for kidney stones and is frequently genetically determined. Certain medications such as calcium-containing antacids, loop diuretics and glucocorticoids can increase calcium secretion into the urine. Too much vitamin D can also lead to increased calcium. Hyperparathyroidism occurs when too much parathyroid hormone is produced by the body, causing calcium to be pulled from the bones into the blood and subsequently into the urine[16]. This helps to explain the association between kidney stones and low bone density. Kidney disease, too, can cause high calcium levels in the urine when calcium is not properly absorbed back into the bloodstream. High blood pressure and obesity have also been associated with hypercalciuria.

High levels of oxalates

Some people are born with a genetic tendency to secrete excess oxalate into the urine. This condition, hyperoxaluria, is rare; most cases of hyperoxaluria arise from other causes. For one, diets rich in oxalate may place someone at risk for kidney stones. Oxalate-rich foods include beets, chocolate, nuts, rhubarb, spinach, strawberries, tea and wheat bran. Excessive amounts of vitamin C can also increase oxalate levels, as can inflammatory bowel disease[17].

High levels of Protein

High amounts of dietary protein can lead to increases in both calcium and oxalate levels in the urine. The elevated protein results in lower urine pH -- an acidic environment that makes it easier for calcium oxalate kidney stones to form. It also decreases citrate levels in the urine that help prevent kidney stones from forming. The risks of kidney stone formation can often be minimized by paying close attention to diet and good hydration[18]. If you are concerned about kidney stones, speak with a health-care provider who can evaluate the type of stones you might have and what dietary changes would be most helpful for you.

Uric acid stones

Uric acid stones are the most common cause of radiolucent kidney stones in children. Several products of purine metabolism are relatively insoluble and can precipitate when urinary pH is low. These include 2- or 8-dihydroxyadenine, adenine, xanthine, and uric acid. The crystals of uric acid may initiate calcium oxalate precipitation in metastable urine concentrates. The terms gouty nephropathy, urate nephropathy, and uric acid nephropathy are used to describe renal insufficiency due to uric acid precipitation within the renal tubules. Uric acid urolithiasis or uric acid kidney stones refer to development of a stone or calculus composed of significant amounts of urate in the renal pelvis, ureter, or bladder.

2.8.COMPLICATION OF KIDNEY STONES

The list of complications that have been mentioned in various sources for Kidney stones includes:

- Haematuria
- Renal colic
- Ureteric obstruction
- Proteinuria
- Dysuria
- Incontinence, urine
- Abdominal pain
- Renal failure

2.9.FACTORS INFLUENCING THE KIDNEY STONES

There are multiple things that cause the formation of renal calculus. By controlling such factors, you can significantly reduce the risk of kidney stones. The reasons that are most commonly associated with the formation of stones are:

Obesity:

Obesity is the mother of several diseases. In addition, it also causes difficulty in the performance of routine life activities. In summer, it becomes unbearable for an obese person to go out in heat. Being overweight is one of the foremost reasons which cause stones to form. A recent study has revealed that more a person weighs the greater is the risk of developing kidney stones.

Hereditary:

The threat of kidney stones lies in the DNA of an individual. For some people this condition runs in their family. It means they are likely to fall prey to it even after taking all the precautionary measures. They are likely to suffer from this condition at some stage in their life.

Diet:

Diet directly influences the health of each and every individual. Diet rich in calcium and salts can lead to the development of stones in the blood purification plant of your body, i.e. the kidney.

Medication:

People taking certain types of medications are also at the risk of forming kidney stones. It happens as a side effect of the drugs. Some medications have this as a known side effect. The pharmaceutical companies mention this thing in the leaflets.

Drinking enough water and taking other necessary precautions is important, when taking those medicines. The people experiencing migraine or seizures use Opiramate

(Topamax). It is one of the drugs which can cause kidney stones.

Supplements:

Supplements have become essential in today's time to keep our bodies healthy. It is also a reason why people have to take poor diet which does not contain all the essential nutrients. However, you should not take too much of a specific nutrient say calcium or Vitamin D. They can result in development of renal calculus.

Medical Conditions:

People with certain medical conditions are also at the risk of kidney stones. For example, those who are already suffering from gout or Crohn's disease are at a higher risk. They are more likely to develop stones in comparison with an otherwise healthy person[19].

Hydration:

Maintaining the hydration of your body is very significant. We all know that 70% of our body is comprised of water. If your water intake is sufficient, you can do away with half of the diseases and also feel fit and fresh.

These reasons increase the risk of kidney stones formation. Thus taking care of all the above causes is paramount, for a happy and a healthy life[20].

2.10.MANAGEMENT OF LITHIASIS

- **MEDICATION BASED ON THE TYPE OF STONES**
- **HERBAL MEDICINES**
- **SURGICAL TREATMENTS FOR KIDNEY STONES**
- **OTHER KIDNEY STONE THERAPIES**

2.10.1.MEDICATION BASED ON THE TYPE OF STONES

The most commonly used medicine for kidney stones to pass the stones from the ureter are

- NSAIDs (Non-steroidal anti-inflammatory drugs)
- Alpha blockers

Calcium stones

Calcium stones are the most regular kind of kidney stone. To avoid them,

Thiazides, Orthophosphateand Potassium citrate were used.

Uric acid stones

Some renal stones are made of uric acid, a waste product that usually exits the body in the urine. To avoid these types of stones,

Potassium citrate, Allopurinol, and sodium bicarbonate were used.

Cystine stones

A very minor number of stones are made of a chemical called cystine. Medications to prevent them include: Penicillamine, Tiopronin andPotassium citrate

Struvite stones

Struvite stones (staghorn calculi) are because of recurrent kidney infections. Antibiotics are used to treat the infection and help to avoid new stones from development. Surgery can be done to exclude the stone. Urease inhibitors are used to prevent struvite stones[21].

Table : 1. ANTI-UROLITHIATIC DRUGS

Name of medication	What it's for	Genaeral information
Thiazide	**Calcium reduction**	**This reduces the amount of calcium in urine and prevents calcium stones**
Sodium cellulose phosphate	**Calcium reduction**	**Binds calcium in the intestine and prevents it from leaking into the urine**
Oral calcium supplement	**Reduces oxalate in the body**	**Prevents absorption of oxalate in to body**
Allupurinol	**Uric acid reduction**	**Reduces uric acid in urine**
Polycitra K	**Uric Acid reduction**	**Prevents uric acid Crystallizing in urine**

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Allopurinol

Allopurinol is a xanthine oxidase inhibitor, prescribed for gout. It is used for the treatment of

high uric acid levels in the urine or blood caused by certain types of cancer chemotherapy.

Trade Names:

Allopurinol (100mg) | Allgout (100mg) |Aloric (100mg) | Lodiric (100mg) |Algor (100mg) | Dynol (100mg) | Myloric (100mg) | Allgoric (100mg)| Kayloric (100mg) Estinol (100mg)

Potassium Citrate

Potassium Citrate is an urinary alkalinizing remedy, prescribed for kidney stones.

Trade Names:

Lasertrate (1100mg/375mg) | Ston-1 (1100mg/375mg/5mL)Ston-1 | (1100mg/375mg/5mL) | Ston-1B6 (1100mg/375mg/20mg) | Ston-1B6 (1100mg/375mg/20mg) | BIO-D3 DSSton-1B6 (714.9mg/263.1mg/15mg)

Tamsulosin

Tamsulosin belongs to alpha blocker, prescribed for benign prostatic hyperplasia (BPH) or prostate enlargement. It increases the flow of urine by soothingthe muscles of the prostate and the lower part of the bladder.

Trade Names:

Veltam (0.2mg) | Flodart | Urimax 0.4 | Gotam | Ubimax (0.4mg) | Uritin | Prostulin (0.4mg) | Veltam (0.4mg) | Tamsin |Prostulin (0.2mg)

Tiopronin

Tiopronin is a chelating agent, prescribed for averting kidney stone formation.

2.10.2.HERBAL MEDICINES

In the therapy of kidney stones, herbs can be used to disrupt them down and avoid their development in the first place. Herbs can also boost the flow of urine and comfort the irritated walls of the urinary tract. Gravelroot has been used as a treatment to dissolve renal stones. The root is usually taken in decoction or tincture base. Hydrangea, madder, and rumex can also assistinbreaking up of renal stones[94]. Aloe vera juice may remove stones and prevent

new ones from development.

Other herbs, such as couch grassand cornsilk, can relax the walls of the urinary tract. Juniper berries are often used to cure urinary tract infections, can be included in a herbal treatment for stones[22].

2.10.3.SURGICAL TREATMENTS FOR KIDNEY STONES

Laser Surgery

If a kidney stone does not step through the ureter within 30 days, surgery is necessary. Urologists use several procedures to break up, remove or bypass kidney stones.

Ureteroscopy

This procedurecan be used to remove or break up (fragment) stones located in the ureter. A special telescopic devicelooks like a long, thin telescope (ureteroscope) is introduced through the urethra and moved through the bladder and up the ureter to the stone. Once the stone is located, the urologist either removes it with a small basket introduced through the ureteroscope (called basket extraction) or breaks the stone with a laser or related device. Ureteroscopy is done under general or regional anesthesia on an outpatient basis.

Lithotripsy

This methodis most operative for stones in the kidney or upper ureter. Lithrotripsy uses an instrument, machine, or probe to breakthe stone into tiny particles that can pass naturally. This technique is not suitable for patients with very large stones or other medical conditions.

Ultrasonic lithotripsy

This technique uses high frequency sound waves supplied through an electronic probe presented into the ureter to disrupt up the kidney stone. The fragments are passed by the patient or removed surgically.

Electrohydraulic lithotripsy (EHL)

This technique involves a flexible probe to disrupt small stones with shock waves produced by electricity. The probe is located close to the stone through a flexible ureteroscope.

Fragments can be passed by the patient or extracted. EHL involves general anesthesia and can be used to disrupt stones anywhere in the urinary system[24].

Extracorporeal shock wave lithotripsy (ESWL)

This technique includes highly focused impulses projected and focused from outside the body to pulverize kidney stones anywhere in the urinary system. The stone usually is reduced to sand-like granules that can be moved out through patient's urine. Large stones may require numerous ESWL treatments. The technique should not generally be used for struvite stones, stones over 1 inch in diameter, or in pregnant women. Patients undergoing lithotripsy are given a sedative and general or regional anesthesia, and the procedure time takes over an hour.

Percutaneous Nephrostolithotomy (PCN)

This surgical technique is done under local anesthesia and intravenous sedation. A guidewire and needle are used to access the inside of the kidney. The surgeon then threads various catheters over the guidewire into the kidney and operates surgical instruments through the catheters to fragment and exclude the kidney stones. This technique achieves a better stone-free outcome in the treatment of medium and large stones than shock wave lithrotripsy. The procedure usually needs hospitalization, and most patients resume normal activity within 2 weeks.

Ureteroscopic

This procedure is done under general anesthesia to treat stones located in the middle and lower ureter. A small, fiberoptic device (ureteroscope) is passed through the urethra and bladder and into the ureter. Small stones are removed and large stones are shattered using a laser or similar device. A small tube (or stent) may be left in the ureter for a few days after treatment to promote healing and prevent blockage from fragments, swelling or spasm.

Open Surgery to Treat Kidney Stones

This procedure needs general anesthesia. An incision is made in the patient's back and the stone is removed through a cut in the ureter or kidney. Most patients need prolonged hospitalization and recovery takes numerous weeks. This practice is now rarely used for kidney stones[25].

2.10.4..Other Kidney Stone Therapies

Ayurvedic remedies for Kidney Stones -- Typical kidney stone therapy involves dietary alterations and herbal therapy.

Bodywork for Kidney Stones -- Reflexology is a technique of bodywork that can stimulate the organs and regulate bodily functions. Treatment can focus on the responses of the thyroid, pituitary gland, and parathyroid glands, spleen, and kidney, among others.

Homeopathy for Renal Stones -- Common therapies can include berberis, magnesia phosphorica and sarsaparilla.

Hydrotherapy for Kidney Stones --Therapies, such as lukewarm sitz baths, can be used to get rid of the pain of kidney stones[26].

2.11.DIAGNOSIS OF LITHIASIS

The various diagnosis test used to determine kidney stones are

Bloodtesting-Blood tests may expose too much calcium or uric acid in blood. Blood test results help screen the health of kidneys.

Urinetesting- The 24-hour urine collection test may show that excreting too many stone-developing minerals or too few stone-avoiding substances. For this test, two urine collections over two successive days were essential.

Imaging- Imaging tests may be used to show kidney stones in your urinary tract. Options range from simple abdominal X-rays, which can miss small kidney stones, to high-speed or dual energy computerized tomography (CT) that may expose even tiny stones. Other imaging techniques include an ultrasound, a noninvasive test, and intravenous urography, which includes injecting dye into an arm vein and taking X-rays (intravenous pyelogram) or attaining CT images (CT urogram) as the dye travels through your kidneys and bladder.

2.12.SYMPTOMS OF LITHIASIS

- causes an infection
- kidney stones can cause pain as they try to pass through
- A persistent ache in the lower back, which is sometimes also felt in the groin , men may have pain in their testicles and scrotum

- nausea (feeling sick)
- A periods of intense pain in the back or side of your abdomen, or occasionally in your groin, which may last for minutes or hours
- pain when you urinate (dysuria)
- feeling restless and unable to lie still
- needing to urinate more often than normal
- blood in your urine (haematuria) this may be caused by the stone scratching the kidney or ureter

The symptoms of a kidney infection are similar to symptoms of kidney stones, but may also include:

- chills and shivering
- A high temperature (fever) of 38C (100.4F)
- diarrhoea
- feeling very weak or tired
- cloudy and bad-smelling urine

2.13.PROPHYLACTIC MEASURES

The best way of avoiding kidney stones is to drink sufficient of water each day to prevent becoming dehydrated. Keeping urine diluted aids to stop waste products getting too concentrated and developing stones. Urine is generally a dark yellow colour in the morning because it comprises a build-up of waste products from the body which has produced overnight. Drinks such as coffee,tea, and fruit juice can count towards fluid consumption, but water is the healthiest choice and is best for avoiding kidney stones formation[87].

Diet : If kidney stone is produced by too much calcium, it is advisable to decrease the amount of oxalates in diet.

Oxalates avoid calcium being absorbed from the body, and can gather in kidney to develop a stone[88].

Foods that includes oxalates:

- asparagus

- berries
- beetroot
- celery
- chocolate
- rhubarb
- leeks
- parsley
- soy products
- almonds, peanuts and cashew nuts
- grains, such as oatmeal, wheat germ and wholewheat

2.14.PATHOPHYSIOLOGY OF CALCIUM OXALATE STONE FORMATION

PHYSIOLOGY

Recent studies using papillary biopsies of stone formers have provided a view of the histology of renal crystal deposition which suggests that the early sequence of events leading to stone formation differs greatly, depending on the type of stone and on the urine chemistry leading to supersaturation[90].

Three general pathways for kidney stone formation are seen:

(1) Stones fixed to the surface of a renal papilla at sites of interstitial apatite plaque (termed Randall's plaque), as seen in idiopathic calcium oxalate stone formers[27];

(2) Stones attached to plugs protruding from the openings of ducts of Bellini, as seen in hyperoxaluria and distal tubular acidosis; and

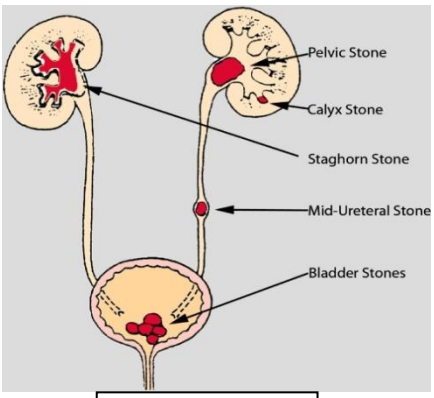


Figure No.5

(3) Stones forming in free solution in the renal collection system, as in cystinuria[91].

The presence of hydroxyapatite crystals in either the interstitial or tubule compartment (and sometimes both) of the renal medulla in stone formers is the rule and has

implications for the initial steps of stone formation and the Location of staghorn and non-staghorn kidney stones.

Staghorn stones fill various amounts of the renal collection system.

Non-staghorn stones can be very variable in size and can be found in a major or minor calyx, in the renal pelvis or at different sites along the ureters (proximal, middle or distal)[28].

Stones can also be found in the urinary bladder

PATHOPHYSIOLOGY OF LITHI'S FORMATION

Mechanism of stone formation[66]

Age	Profession	Nutrition	Climate	Inheritance
Sex	Mentality	Constitutions	Race	-------

↓

Abnormal renal morphology Disturbed urine flow	Urinary tract infection	Metabolic Abnormalities	Genetic Factors

↓

Increased excretion stone forming constituents	Decreased excretion of inhibitors of Crystallizations

↓

Physico-chemical change in the State of supersaturation

↓

Abnormal crystalluria
Crystals aggregation
Crystal growth

↓

Formation of stone

Hypotheses of stone formation and growth

Fixed and free particle theories

Kidney stone development is thought to require the formation of crystals in the tubular fluid, followed by crystal retention and accumulation in the kidney [29].

Three pathways of stone formation and growth are currently being investigated .

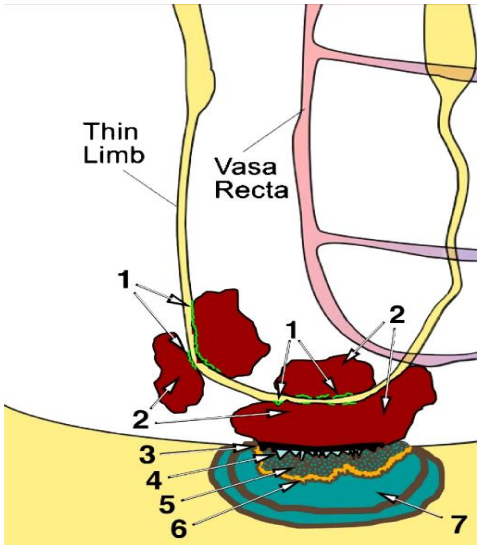
The first hypothesis, termed the free particle model, states that crystal nuclei form by homogeneous nucleation in the lumen of the nephron under conditions of a phase change (increasing supersaturation) in the dissolved salts present in the ultrafiltrate [30]. Subsequently, these nuclei would grow in size and eventually lodge (be retained) in the lumen of the distal nephron, causing obstruction of that tubular segment. Obviously, free particle formation could occur in the renal collection system at the level of the minor calyx[92].

The second hypothesis, termed the fixed particle model, also requires crystal nuclei to form in the lumen of the nephron, and then adhere to the apical surface of the tubular epithelium [31]. While a number of mechanisms have been proposed to model this crystal–cell attachment step, the most commonly cited model requires renal cell injury, probably as a result of high tubular oxalate levels [32]. Once the crystal–cell attachment step has occurred, the crystal nuclei would be fixed in position and exposed to the potentially supersaturated ultrafiltrate that would facilitate further growth of these crystals.

Both these theories could result in the plugging of the nephron and lead to intratubular calcification, termed tubular nephrocalcinosis [33]. Randall, in his historic paper in 1940, described intraluminal calcification (papillary lesion type II) or nephrocalcinosis in only 23 cases and compared it to the more common finding (204 cases) of interstitial plaque (papillary lesion type I), placing the type II lesion in a category different from that of type I [34].

We have shown that, in patients who form brushite stones [35], or who form apatite [hydroxyapatite, $Ca_5(PO_4)_3(OH)$] stones because of distal renal tubular acidosis (dRTA) [36], or patients with calcium oxalate stone due to obesity bypass procedures [37], or patients with

cystinuria [38], their inner medullary collecting ducts (IMCDs) are plugged (tubular nephrocalcinosis) with cystine, which leads to total destruction of the lining cells and focal sites of interstitial fibrosis. However, it is unclear how the free or fixed particle hypotheses could lead to clinical stone formation (i.e. a stone in the renal pelvis large enough to obstruct the ureter), except that either the free or fixed particle process, or both, are occurring in the lumen of the ducts of Bellini and/or in the renal pelvis.



Schematic representation of stone development in idiopathic calcium oxalate stone formers. The sequence of steps are as follows: *1* apatite deposits develop in the basement membrane of the thin loops of Henle; *2* these apatite deposits further extends.

Figure No.6

The pathogenesis of calcium oxalate stone formation is a multi-step process and in essence includes – nucleation, crystal growth, crystal aggregation and crystal retention. Various substances in the body have an effect on one or more of the above stone forming processes, thereby influencing a person's ability to promote or prevent stone formation[93].

Promoters of stone formation facilitate stone formation whilst inhibitors prevent it.

- Low urine volumelow urine pH
- Calcium
- Sodium
- Oxalate and
- urate

Many inorganic (eg.Citrate, magnesium) and organic (eg. Urinary prothrombin fragment 1, glycosaminoglycans, osteopontin) substances are known to inhibit stone formation.

Organic inhibitory compounds adsorb to the surface of the crystal, thereby inhibiting crystal growth and nucleation. This review presents a comprehensive account of the basic principles of stone formation and role of urinary inhibitors/promoters in calcium oxalate crystallisation.[63]

2.15.MODES OF STONE GROWTH

The modes and putative sites of stone formation are depicted in Figure 6.

Nucleation

Nucleation is the process by which free ions in solution associate into microscopic particles. Crystallization can occur in solution micro-environments, such as may be present in certain points in the nephron [64], as well as on surfaces, such as those of cells and on extracellular matrix [68]. There is considerable dispute about the importance of free solution crystallization versus crystallization at other sites, in renal tubules or on bladder walls, on normal or damaged cells, on areas denuded of cells by certain forms of injury, or at interstitial sites.[69]

Aggregation

Aggregation is a process by which there is agglomeration of crystals that form in free solution into larger multicomponent particles. It may also encompass the phenomenon of secondary nucleation of new crystals on the surface of those already formed. The structure of stones suggests that one or other of these processes must occur for the stone to grow to a clinically significant size.[70] Kidney stones can be thought of as being similar to concrete, a mixture of a binding agent (cement), and particulates such as sand, pebbles, or glass. Stones are an aggregation of crystals and an organic matrix, the latter serving as the binding agent. The organic matrix contains proteins, lipids, polysaccharides, and other cell-derived material.[71]

Crystal growth

Growth of microscopic crystals is accomplished by movement of ions out of solution onto the growing crystal. While some growth of nuclear crystals must occur by movement of ions from solution, this is clearly a limited process, as giant single crystals of stone constituents are not generally observed. It is more likely that stone growth is accomplished through aggregation of preformed crystals or secondary nucleation of crystal on the matrix coated surface of another[95]. It has been proposed that the growth of these microscopic crystals to the extent that they can be retained in the kidney on the basis of size alone cannot occur without aggregation or attachment to specific intrarenal structures.[39]

2.16.PROMOTERS OF STONE FORMATION

Promoters can increase crystallization of stone constituents or their growth by a number of mechanisms. The saturation can be raised by increases in concentration of the reactants. Substances in urine may be present that lower the formation product, but the formation product may also be lowered by a lack of endogenous inhibitors or opposition to their effects by defects in their structure or other interfering substances. Excessively low or high urinary pH may induce the formation of heterogeneous nucleating substances[65].

Uric acid or urate

Monosodium urate appears to directly reduce the formation product for calcium oxalate.[40] The mechanism for this effect is likely through its antagonistic effecV t to substances in the urine that raise the formation product, specifically that attributable to mucopolysaccharides. [41]In addition to this effect there may be promotion of heterogeneous nucleation by uric acid or monosodium urate and enhancement of the attachment of calcium oxalate to cells.[42]

Urine pH

Highly acid urine leading to precipitation of uric acid crystals may not only lead to uric acid stone disease but may also enhance calcium oxalate crystallization due to heterogeneous crystallization, in which one type of crystal acts as a template, thereby promoting crystallization of a second type of crystal, as noted above.[43] Highly alkaline urine may also promote secondary nucleation of calcium oxalate by precipitation of calcium phosphate.[44]

Low pH cannot really be said to promote crystallization of cystine, as the solubility of this substance is minimal at most usual urinary pH values. On the other hand, the solubility of this substance, increases significantly at pH values in excess of 7.5.[45] Commercial laboratories that market packages of urine stone chemistries consider stone risk to be minimal when urine pH is between 5.8–6.2, in one instance, or between 5.5–7.0 in another.

2.17.INHIBITORS OF STONE FORMATION

There are at least four types of inhibitors in urine: small organic anions such as citrate, small inorganic anions such as pyrophosphates, multivalent metallic cations such as magnesium, or macromolecules such as osteopontin and Tamm-Horsfall protein.

Alkaline pH

Alkaline pH cannot be said to be an inhibitor of stone formation, as it has both beneficial and deleterious effects depending on the stone constituent under consideration, however alkaline pH inhibits cystine and uric acid stone formation, which tend to form in acidic urine, as noted above.

Citrate

Citrate can be said to be an inhibitor of stone formation. It has several effects. It will lower saturation of calcium oxalate by virtue of forming complexes with calcium. When studies are performed in which free calcium is controlled, it appears to have an independent effect of nucleation and growth (unpublished observations). However, it has also been shown to inhibit aggregation of preformed crystals as well as attachment of crystals to urinary epithelium.[46]

Pyrophosphate

Pyrophosphate, a naturally occurring substance in urine, has been demonstrated to inhibit both calcium oxalate and calcium phosphate crystallization. It was found that the average urine pyrophosphate concentration was sufficient to significantly inhibit crystal growth. [47] This agent has given discordant results on tests of aggregation and crystal attachment to epithelia.[48]

Phytate

Phytate (myo-inositol hexakisphosphate), a natural compound formed during maturation of plant seeds and grains is a common constituent of plant-derived foods. In an animal model, calcification in renal tissue was induced in hypercalcemic, hypertensive, male Wistar rats that were fed a purified phytate-free diet.[49] On this diet rats developed significant calcium deposits in kidneys and papillae, as well as in kidney tubules and vessels, whereas calcium deposits were absent in control and phytate treated rats. Fragments of hydroxyapatite (HAP) calculi exhibited the capacity to induce the growth of calcium salts on their surfaces, however 1.5 mg/L phytate in the synthetic urine utilized in the study inhibited the formation of calcium oxalate monohydrate on HAP renal calculi fragments under when calcium concentration were in the range considered normal. These findings show that the action of phytate as a crystallization inhibitor takes place both in the intrapapillary tissue and urine[50].

Magnesium

Magnesium has also been demonstrated to inhibit stone formation by inhibition of growth (and presumably nucleation) of crystals as well as aggregation. Inhibition of crystal attachment of calcium oxalate appears to require supra-physiologic concentrations.[51] Stone formation in vitamin B6-deficient animals has been attributed to magnesium depletion, as it is ameliorated by magnesium repletion.[52]However, magnesium supplementation for stone prevention in humans has had disappointing results.[53]

Glycoproteins

The effects of the small amount of proteins and glycosaminoglycans present in urine are more complex. A number of them are found preferentially in stone matrix, specifically, osteopontin/uropontin, Tamm-Horsfall protein, urinary prothrombin fragment 1, and some subunits of the serum inter-α-inhibitor. As noted earlier, some of these substances may act as attachment sites—hence promoters—when expressed on the surface of cells. As will be explained below, the physico-chemical state of these substances may also determine whether they act as inhibitors of stone formation processes or promoters. Finally, it is to be expected that proteomic analysis of stone matrix may reveal other stone matrix components involved in stone formation, however, to date there are little published data[54].

Osteopontin/uropontin

Osteopontin/uropontin inhibits spontaneous nucleation from metastable solutions as well as the growth of preformed crystals in a seed growth assay.[55] The intact molecule and several regions associated with both acidic amino acid residues and phosphorylation slows crystal growth in seeded assay systems and the intact molecule inhibits attachment to cells, at least in some reports.[56] Others have provided evidence that osteopontin/uropontin bound to the surface of cells may enhance attachment.[56] And finally, calcium oxalate aggregation inhibition by osteopontin *in vitro* can be switched to aggregation promotion by neutralization of its net negative charge by poly-arginine.[57]

3.OBJECTIVE OF THE STUDY

The problem of urinary stone or calculi is a very ancient one . The stones form in all parts of the urinary tract , the kidneys, the ureters and in bladders and they may vary in size considerably .

Majority of calculi are made up of Calcium oxalate, calcium phosphate, uric acid or magnesium ammonium phosphate. In India the common components of urinary calculi is calcium oxalate. A number of vegetable drugs have been used in India and elsewhereand gain efficient cure of urinary stones[58].

There are several options available in the management of ureteral stones. Treatment depends upon the size of the stones, composition and associated morbidity, physician skill, patient health ,preference and finally costs[59]

Many remedies have been employed during the ages for treating urolithiasis. Most of the remedies were taken from plants proved to be useful, though the rational behind their use is not scientifically established except for a few plants and some proprietary composite herbal drugs.[60]

3.1.SCOPE AND PLAN OF WORK

3.1 Scope of work

Recently there has been increasing interest in use of traditional medicine .In many countries trditional medicines used for controlling kidney disorders significantly there is a increase in occurrence of kidney stones in recent years.Therefor they look for more effective and safer antiurolithiatic agents

Estimated life time risk of 2%-5% in Asia , 8%-15% in Europe and America and around 20% in Middle East it is associated with a high risk of re-occurence .Nephrolithiasis is a frequent disease that affects about 10% of people in western countries. The prevalence of calcium oxalate stones has been constantly increasing during the past fifty years Stone composition varies depending to gender and age of patients and also underlines the role of other risk

factors and associated pathologies such as body mass index and diabetes mellitus.A high prevalence of uric acid was found in overweight and obese stone formers and in diabetic ones as well. Another important finding was the increased occurrence with time of calcium oxalate stones formed from papillary Randall's plaques, especially in young patients. Nutritional risk factors for stone disease are well known: they include excessive consumption of animal proteins, sodium chloride and rapidly absorbed glucides, and insufficient dietary intake of fruits and potassium-rich vegetables, which provide an alkaline load. As a consequence, an excessive production of hydrogen ions may induce several urinary disorders including low urine pH, high urine calcium and uric acid excretion and low urine citrate excretion.[76] Excess in calorie intake, high chocolate consumption inducing hyperoxaluria and low water intake are other factors, which favour excessive urine concentration of solutes. Restoring the dietary balance is the first advice to prevent stone recurrence. However, the striking increase of some types of calculi, such as calcium oxalate stones developed from Randall's plaque, should alert to peculiar lithogenetic risk factors and suggests that specific advices should be given to prevent stone formation.

Urolithiasis is a global problem , successful treatment is very important for preventing or atleast delaying the onset of long term complications. When compared to that of the synthetically derived drugs , medicines derived from the natural medicinal plants possess lesser side effects .Therefore the search for safer and more effective antiurolithiatic agents has become an area of active research, it is also believed that the traditional medicine attenuate the progression of complications of the disease[77]

Traditionally *apium graveolens* seeds are used to treat Kidney stone.The objective of this study was to ascertain the scientific basis for the use of this *Apium graveolens* in the management of Kidney stones using ethylene glycol induced urolithiasis in rats .

Apium graveolens used in the traditional system of medicine in India for the treatment of Kidney stones of pharmacologically evaluate the plant for its antinephrolithiatic property potential in the present study

PLAN OF WORK

1. **COLLECTION AND AUTHENTICATION OF PLANT**
2. **PREPARATION OF EXTRACT**
3. **PHYTO CHEMICAL SCREENING**
4. **ACUTE ORAL TOXICITY STUDY**

- **ASSESSMENT OF UROLITHIATIC ACTIVITY**

1. **Measurement of body weight**
2. **Measurement of food intake**
3. **Measurement of water intake**
4. **Measurement of urine volume**
5. **Collection and analysis of urine**

- **Calcium**
- **Magnesium**
- **Phosphate**

6.Analysis of serum

- **Serum creatinine**
- **SGOT**
- **SGPT**
- **Serum Urea**
- **Uric acid**

- **HISTOPATHOLOGICAL STUDIES**
- **DATA INTERPRETATION**
- **STATISTICAL ANALYSIS**

4.MATERIALS AND METHODS

4.1.LITERATURE REVIEW OF PLANT

1.Antinociceptive effects of isolated fractions from *Apium graveolens* seeds

Mina Ramezani et al. 2009 Jun 12 47(8)

The antinociceptive effects of the aqueous and hexane extracts obtained from *Apium graveolens* L. (Apiaceae) seeds were evaluated. the hexane fraction reduced the nociception produced by formalin solution in the first phase (0-5 min) at 300, 400, and 500 mg/kg BW, and in the second phase (20-30 min) at 500 mg/kg BW. It is concluded that the hexane fraction has major contribution to the overall antinociceptive activity. Both fractions showed remarkable anti-nociceptive effect which supported the traditional use of *Apium graveolens* in diseases associated with inflammation.

2.The Anti-inflammatory Activity of Celery *Apium graveolens* L. (Fam. Umbelliferae)

DA Lewis et al. 2008 Sep 27 23(1)

Aqueous extracts of celery stem have been investigated and found to have significant anti-inflammatory activity against two animal models. Celery contained a phytosterol with some anti-inflammatory activity but it was concluded that the major anti-inflammatory effect was due to unidentified polar substances. Mannitol was also identified in celery but this compound did not reduce carrageenan-induced oedema in the rat although the celery fraction with anti-inflammatory action was active against this model. Mannitol has been reported to reduce inflammation in adjuvant-induced arthritis in the rat. It was concluded that celery stem does possess anti-inflammatory properties which may form a basis for the reputation of the plant as a medicinal treatment for rheumatic disease.

3. Hepatoprotective activity of *Apium graveolens* and *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats

Anuba Singh et al. 1995 Dec 15 119-126

Seeds of *Apium graveolens* L. (Apiaceae) and *Hygrophila auriculata* (K. Schum.) Heine (Syn. *Astercantha auriculata* Nees, Acanthaceae) are used in Indian systems of medicine for the treatment of liver ailments. The antihepatotoxic effect of methanolic extracts of the seeds of these two plants was studied on rat liver damage induced by a single dose of paracetamol (3 g/kg p.o.) or thioacetamide (100 mg/kg, s.c.) by monitoring several liver function tests, viz. serum transaminases (SGOT and SGPT), alkaline phosphatase, sorbitol dehydrogenase, glutamate dehydrogenase and bilirubin in serum. Furthermore, hepatic tissues were processed for assay of triglycerides and histopathological alterations simultaneously. A significant hepatoprotective activity of the methanolic extract of the seeds of both the plants was reported.

4. In vitro antimicrobial, antioxidant activity and phytochemical screening of Apium graveolens.

Zakir Ud Din et al. 2015 Sep 5 Pg-1699-1704

The present study evaluates the phytochemical screenings, antioxidant activity and antimicrobial assay of Apium graveolens L. The phytochemical screening showed the presence of flavonoids, tannins, saponins and steroids in Apium graveolens while terpenoids was absent. The total phenolic content was slightly higher in methanolic fraction (63.46 ± 12.00 mg GAE/g) followed by ethanol (36.60 ± 12.28 mg GAE/g) and hexane fractions (34.86 ± 6.96 mg GAE/g). The flavonoid content was high in methanolic extract (56.95 ± 7.14 mg Quorcetin/g) and low level of the content was found in methylated spirit extract (29.2 ± 3.15 mg Quercitin/g). Antioxidant activity assayed by FRAP was higher in methanolic fraction (12.48 ± 1.06 mmole of $FeSO_4$equivalent/litre of extract) compared with other extracts. Likewise, good antimicrobial activity was measured by crude ethanol fraction against S. aureus (MIC=0.12 ± 0.03 µg/ml) and S. typhi (MIC= 0.5 ± 0.2 µg/ml). Results also that ethanolic fraction was effective against A. flavus (MIC= 05 ± 1.0 µg/ml).

5.Vasorelaxant activity of extracts obtained from *Apium graveolens*: Possible source for vasorelaxant molecules isolation with potential antihypertensive effect

Vergara-GaliciaJorgeet al.2013 October 10 Pg 776-779

To investigate vasorelaxant effect of organic extracts from *Apium graveolens (A. graveolens)* which is a part of a group of plants subjected to pharmacological and phytochemical study with the purpose of offering it as an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects.An *ex vivo* method was employed to assess the vasorelaxant activity. This consisted of using rat aortic rings with and without endothelium precontracted with norepinephrine.All extracts caused concentration-dependent relaxation in precontracted aortic rings with and without endothelium; the most active extracts were Dichloromethane and Ethyl Acetate extracts from *A. graveolens*. These results suggested that secondary metabolites responsible for the vasorelaxant activity belong to a group of compounds of medium polarity. Also, our evidence showed that effect induced by dichloromethane and ethyl acetate extracts from *A. graveolens* is mediated probably by calcium antagonism.*A. graveolens* represents an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects.

6.Application of *Apium graveolens* in treatment of hypertension

Gharouni Manocheher et al. 2000 Vol 58 Pg 67-69

Apium graveolence has an small brown olyptical seed with pharmacological activity. To evaluate it's application in treatment of hypertension, 37 hypertensive patients (20 Female, 17 male) with the age range of 45-65 were given 6 grams of powder of apium graveolence seed and then the blood pressures before end after the remedy were compared. Before treatment, the plean systolic blood pressures was 171/35mlHg and the mean diastolic blood pressure was 94 mlHg. After they became 154/3mlHg and 89/6mIHg respectively.The difference of blood pressure before and after treatment was statistically significant ($P<0.05$), so we concluded that apium graveolence seed can he used as is a safe and effective treatment for high blood pressure. Further controlled studies should be done to compare it with available antihypertensive drugs.

Part-a

- **Plant Collection and authentication**
- **Plant Introduction**
- **Sample preparation and extraction**
- **List of chemicals used**
- **Preliminary phytochemical analysis**

4.2.PLANT COLLECTION AND AUTHENTICATION

The seeds of *Apium graveolens L* (apiaceae) were collected from local source, Tamil Nadu (Chennai) in the month of March. The seeds were identified and authenticated by Prof. P.Jayaraman, Retd Professor of Presidency College and Director of Institute of Herbal Science Plant Anatomy Research Centre, West Tambaram, Chennai-600045. A voucher specimen was deposited at C.L.Baid Metha College of Pharmacy for future reference.

4.3.PLANT INTRODUCTION

APIUM GRAVEOLENS

The scientific name of celery, celery seed is *Apium Graveolens*. It is also known with the names of marsh parsley and wild celery. In Hindi it is called Ajwain ka patta. Celery has been used in Chinese medicine since fifth century BC onwards. It is a salad plant.

In India celery is used in Ayurvedic medicine to relieve flu, colds, poor digestion, water retention, disorders of spleen and liver and different types of arthritis. The volatile oil is used in pharmaceutical and perfume industries[61].Apium graveolens Linn. (Apiaceae) is commonly known as Celery (Norman et al., 2001). In India Celery plant is native in Punjab, Himachal Pradesh and Uttar Pradesh states. It is commonly found in foot hills of Himalayas (Pullaiah, 2006; Nadkarni and Nadkarni, 1976; Singh et al., 1995).

Figure No.7 – Apium graveolens seeds

SYNONYMS

Apium graveolens .var dulce

Apium graveolens L

Apium petroselinum Linn

Apium graveolens .var rapaceum

CHARACTERISTIC OF *APIUM GRAVEOLENS*

An erect ,annual or biennial herb with very small seedsCelery plant is thin and grows to a height of two to three feet. It has around three to five segmented flowers and leaves. The flowers are green-white in colour and the seeds are grayish brown in colour. It is biennial aromatic plant which has fleshy bulbous roots.

DESCRIPTION

It is an erect, annual or biennial herb. The roots are numerous, succulent and well developed. The stem branches are angular or fistular, and are conspicuously jointed. The leaves are oblong to obovate, pinnate or trifoliolate. The leaflets are ovate to suborbicular and 3-lobed. The flowers are white or greenish white and very small. The fruit is a schizocarp consisting of two mericarps, sub-orbicular to ellipsoid, greyish brown to brown with pale ridges, aromatic and slightly bitter. The seed and flowering shoots[62]

Figure No. 8

TAXONOMY

Domain : Eukaryota

Kingdom : Plantae

Phylum : Spermatophyta

Class : Dicotyledonae

Order : Apailes

Family : Apiaceae

Genus : Apium

Species : Apium graveolens

VERNACULAR NAMES

English name : Celery , Ajowan

Indian : Ajamoda

Tamil : Seevarikeerai , callari

Hindi : Shalari

Arabic : Ajmod

USES

Internal Use :

- It is an aromatic bitter herb which reduces blood pressure, relieves indigestion, and stimulates the uterus, acts as anti-inflammatory, diuretic and aphrodisiac.
- Celery contains furocoumarins which are used for its carminative, stomachic, emmenagogue and diuretic properties.
- It is taken internally for relieving rheumatoid arthritis, osteoarthritis, gout and inflamed urinary tract.
- In Ayurveda it is used as a nerve tonic and also to relieve bronchitis and asthma.

External use :

- Celery has been used for relieving fungal infections and also tumours.

Aromatherapy :

Seeds are used for distilling oil and it is useful in toning the nervous system, relieving cellulite and water retention. The essential oil of celery cleanses and purifies the kidneys, liver and spleen. It helps to reduce uric acid in the joints of arthritis, rheumatic and gout patients. The puffiness of the skin is reduced with the usage of celery essential oil.

ECONOMIC IMPORTANCE

THIS HERB PROVES HELPFUL IN RELIEVING SEVERAL AILMENTS. SOME OF THESE ARE:

- **Cancer:** The seeds of celery have cancer fighting abilities. There are some active components which are helpful in combating cancer tumours like phthalides and polyacetylenes.
- **Blood pressure:** Consuming celery seeds everyday will promote optimum levels of blood pressure.
- **Liver disorders**: Negative effects of acetaminophen are reduced with the usage of celery seeds. Liver is protected because of the analgesic properties.
- **Cholesterol:** Celery seeds have the ability to lessen cholesterol levels. Herbal practitioners advocate the usage of celery seeds for promoting circulatory system.
- **Pain and inflammation:** Because of the anti-inflammatory agents in celery, it acts well in relieving inflammation and pain of the joints. These seeds work well on ailments like rheumatism, gout and arthritis.
- **Muscle spasms**: Celery seeds are stimulating in nature and help to ease the uterus muscles during menstruation. Women can get relief from menstrual cramps and pains by using celery seeds.
- **Kidney problems:** Celery seeds are diuretic and hence eliminate excess uric acid from the body. These seeds can be consumed by people suffering from gout, water retention and kidney stones.

- **Urinary tract infections:** Celery seeds have antiseptic and antibiotic properties and can be used to relieve urinary tract infections. Patients suffering from bladder ailments, cystitis and kidney problems are advised to use celery seeds every day.

PHARMACOLOGICAL ACTIVITIES

- Celery is said to promote the elimination of uric acid. It is an aromatic bitter herb that reduces blood pressure, relieves indigestion, stimulates the uterus and act as anti-inflammatory (Leung et al., 1980).
- The ripe seeds, herb and root are uricosuric, nervine, stimulant and tonic (Zheng et al., 1993; Ko, 1991).
- An essential oil obtained from the plant has a calming effect on the central nervous system
- Some of its constituents have antispasmodic, sedative and anticonvulsant actions.
- It has been shown to be of value in treating high blood pressure (Bisset et al., 1994).
- It is used in treating rheumatism and kidney complaints (Yan et al., 1998).
- It is considered one of the most alkaline of foods.
- It has a special affinity for the stomach, kidneys, and liver and helps to neutralize acids in the body.

4.4.SAMPLE PREPARATION AND EXTRACTION

Apium graveolens seeds were collected and washed with clean water. It was dried at room temperature for a week. Then it was powdered using an electronic mixer. The powdered drug was extracted by continuous solvent extraction method with Soxhlet apparatus. The ethanolic extract was concentrated by solvent evaporation. 5gm of the crude drug sample was taken in a extractor tube and a mixture of 50 ml ethanol with 50 ml water was added to it. After the completion of 30 cycles the extract was removed. The percentage yield of ethanolic extract of Apium graveolens was found to be 7%. The extract was subjected to Hexane for defatting. The concentrated crude extracts were stored at 4°C in a refrigerator and used for further study

4.5.LIST OF CHEMICALS USED

S.NO	MATERIAL	SOURCE
1	Ethylene glycol	S.d.fine chemicals Ltd. Mumbai
2	Formalin 10%	S.d.fine chemicals Ltd. Mumbai
3	Chloroform L.R	S.d.fine chemicals Ltd. Mumbai
4	Diethyl ether L.R	S.d.fine chemicals Ltd. Mumbai
5	Ammonium Chloride	S.d.fine chemicals Ltd. Mumbai
6	Ethanol	S.d.fine chemicals Ltd. Mumbai
7	Concentrated HCL	S.d.fine chemicals Ltd. Mumbai
8	Calcium kit	S.d.fine chemicals Ltd. Mumbai
9	Nitric oxide kit	S.d.fine chemicals Ltd. Mumbai
10	Phosphate kit	S.d.fine chemicals Ltd. Mumbai
11	Creatinine kit	S.d.fine chemicals Ltd. Mumbai
12	Thiazide	Ranbaxy Laboratories Pvt Limited N.Delhi
13	Ethylene diamine tetra acetic acid (EDTA) Disodium Salt	Chemspure, Chennai
14	Hydrogen peroxide	Chemspure, Chennai
15	Distilled water	Andavar Distilled Water Company, Chennai
16	Sodium hydroxide	Chemspure, Chennai
17	Sodium bicarbonate	S.d.fine chemicals Ltd. Mumbai

4.6.PHYTOCHEMICAL INVESTIGATION

Materials and methods :

Chemicals and reagents:

Laboratory grade chemicals were used for routine work . Analytical grade reagents (A.R) were used for analytical work .

Preliminary phytochemical screening

Ethanolic seed extract of apium graveolens were subjected to phytochemical screening for the presence or absence of phytoconstituents by the following methods.

- **Test for alkaloids**

The extract was treated with dilute hydrochloric acid and filtered . The filtrate is used in the following tests.

a) Mayer's reagent (Potassium Mercuric Iodine solution)

0.5 ml the extract was treated with Mayer's reagent and the appearance of cream colour indicates the presence of alkaloid

b) Dragendroff's test (Potassium Bismuth iodide)

0.5 ml the extract was treated with Dragendroff's reagent and the appearance of reddish brown colour indicates the presence of alkaloid

Test for alkaloids: The extract was treated with dilute hydro chloric acid and filtered. The filtrate is used in the following tests. • Mayer's reagent (Potassium Mercuric Iodine Solution) 0.5 ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid • Dragendroff's test (Potassium Bismuth Iodide) 0.5ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown colour precipitate indicates the presence of alkaloid.

c)Wagner's test (Iodine-Potassium Iodide Solution)

0.5 ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

Test for Carbohydrates

• Molisch's test

The extract was treated with 3 ml of alpha-naptholin alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates.

• Fehling's test (CuSO4.7H2O +KOH+ Potassium Tartartes)

The extract was treated with Fehling's solution A and B heated in boiling water for few minutes. The appearance of reddish brown colour precipitate indicates the presence of reducing sugars.

• Benedict's test (Sodium citrate + sodium carbonate + CuSO4.7H2O)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange colour precipitate indicates the presence of reducing sugars.

Test for steroids

• Liberamann burchard test

The extract was treated with small quantity of concentrated sulphuric acid, glacial acetic acid and acetic anhydride. The appearance of green colour indicates the presence of steroids

• Salkowsi test

The extract was treated with chloroform and to that add a drop of concentrated sulphuric acid. The appearance of golden yellow colour was obtained.

Test for proteins

• Millon's test

The extract was treated with Millon's reagent. The appearance of pink colour indicates the presence of proteins.

Test for Tannins

Lead acetate test

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins

b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

Test for Phenols

• Ferric chloride test

The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.

• Liebermann test

The extract was treated with sodium nitrite and add a drop of concentrated sulphuric acid. The appearance of red colour after turns to black colour was obtained.

• Lead acetate test

The extract was treated with 10 ml water and 5 drops of 1% lead acetate solution was added. The appearance of white precipitate was obtained.

Test for Flavonoids

5 ml of extract solution was hydrolysed with 10% v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes.1 ml of diluted sodium carbonate, 1 ml of 0.1N sodium hydroxide, and1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow colour demonstrated the presence of flavonoids.

Test for Glycosides

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides

Test for Saponins

1ml of the extract was diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

Test for Terpenoids

The extract was treated with tin and thionyl chloride. The appearance of pink colour indicates the presence of terpenoids..

Test for sterols

The extract was treated with 5% potassium hydroxide solution; appearance of pink colour indicates the presence of sterols. Test for thiol: The extract is treated with ellman reagents A and B. The appearance of yellow colour solution was obtained

PART –B

5.Animal Studies

5.1.Experimental animals

33 Male Albino Wistar rats (150-350 g) were used in the present experimental study.[75]All the experimental procedures and protocols used in this study were approved from the Institutional Animal Ethics Committee (/IAEC/L/01/CLBMCP/2017).

The animals were kept in polypropylene cages and maintained under standardized conditions (temperature 27°C ± 1°C, humidity 60% ± 4%, and natural lighting), fed with a standard diet and water. The rats were divided into five groups, a minimum of six animals were used in each group.They were housed five per cage under standard laboratory conditions at a room temperature at 22±20C with 12hr light/dark cycle. The animals were provided with pellet chow and water. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

IAEC Reference no: IAEC//L/01/CLBMCP/2017

- Species / Common name :Wistar Albino rats
- weight : 150g-350 g
- Gender : Male

30 male animals were used in the study of Nephrolithiasis and 3 female animals were used for acute toxicity study

Grouping of animals

S.NO	GROUPING	ANIMALS REQUIRED
1	Group I -CONTROL	6 male Wistar Albino rats
2	Group II – NEGATIVE CONTROL	6 male Wistar Albino rats
3	Group III - STANDARD	6 male Wistar Albino rats
4	GroupIV-LOW DOSE(EEGA 200mg/kg)	6 male Wistar Albino rats
5	GroupV-HIGH DOSE(EEGA 400mg/kg)	6 male Wistar Albino rats

Experimental Procedure

30 healthy male adult Wistar rats weighing (200-250g) were divided into 5 groups consisting of six animals in each group. Ethylene glycol and ammonium chloride induced urolithiatic model in rats was used to assess the effect of ethanolic extract of *Apium graveolens.*

Rats were housed in cages for entire duration of the experiment. The urine of each rat was collected on the 28th day of the experiment. The estimation of biochemical parameters like Urea, creatinine, SGOT, SGPT, Uric acid were carried out.

Administration of dose

Stones were induced in rats by giving 0.75% of ethylene glycol and 1% of ammonium chloride in 100ml of drinking water per day orally. The inducing agent was administered for 28 days In the curative study two groups have involved Low dose (200mg/kg) and High dose (400mg/kg) all the groups except control, standard and negative control received extract by oral route from 15^{th} day till 28^{th} day[74]

5.2.ACUTE ORAL TOXICITY STUDY

The acute oral toxicity study was carried out as per the guidelines set by the organization for economic co-operation and development (OECD) revised draft guidelines 423B received from the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of social Justice and Empowerment, Government of India .

The LD-50 cut-off dose for EtAG of seeds are given in Table 11of the LD 50 dose was taken as a therapeutic doseAcute oral toxicity study was performed as per guidelines [72] Albino mice (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting overnight providing only water, after which the extract (50% ethanolic extract) was administered orally at the dose level of 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose would be repeated again to confirm the toxic dose. If mortality was not observed, the procedure was carried out accordingly. 2000 mg/kg body weight was administered. According to the results of the acute toxicity test, the doses were chosen for experiments.

EXPERIMENTAL PROCEDURE

Healthy adult wistar albino rats weighing 150-200 grams were used for the study, The starting dose level of 2000mg/kg body weight p.o of *Apium graveolens* was given. Since most of the crude extracts possess LD50 value more than 2000 mg/kg, p.o. so starting dose 2000mg/g p.o. was used. Dose volume administered was 1ml/100 gm body weight to each rat which werefasted overnight with water *ad libitum*. Food was withheld for further 3-4hrs after oral administration of drugs and observed for the signs of toxicity.

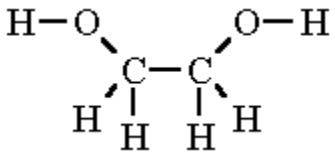
Body weight of each rat before and after administration of *Apium graveolens* was noted and any changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic& central nervous system, motor activity and behavior pattern was observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma was noted. The onset of toxicity and signs of toxicity was also noted. The animals were kept under observation for 14 days[73]

STONE INDUCTION METHOD

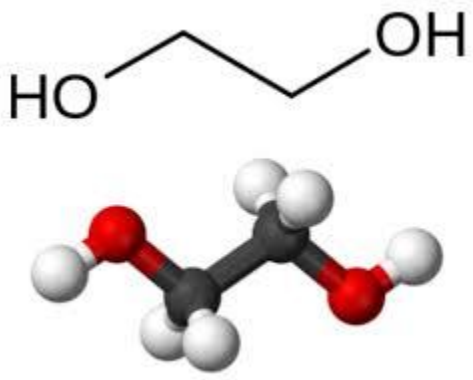
Animals receiving 0.75% v/v EG and 1%v/v NH^4CL was added in drinking water for 28 days forming renal calculi composed mainly of calcium oxalate

6.1.Ethylene Glycol :

Structure of ethylene glycol



Ethyelene glycol is widely used for kidney stone formation in rats. When ethylene glycol is metabolized by the body , it produces three toxic metabolites : Glycoaldehyde, glycolate and glyoxylate , these metabolites causes tissue destruction , primarily from calcium oxalate crystal deposition, and metabolic abnormalities , speacially a high anion gap metabolic acidosis, lactic acidosis and hypocalcemia . Oxalic acid combines with calcium to form calcium oxalate crystals, which deposit in the kidneys



The accumulation of Calcium Oxalate Monohydrate(COM) crystals in kidney tissue produces renal tubular necrosis that leads to kidney failure . In vivo models in EG –dosed rats have correlated the severity of renal damage with the accumulation of COM crystals in kidney tissue . In cultured kidney cells, including human Proximal tubule (HPT) cells , have demonstrated that only the COM crystals , not the oxalae ion, glycoldehyde , or glyoxylate , produce a necrotic cell death at toxicologically relevant concentrations. In EG poisoning ,

COM crystals accumulate to higher concentrations in the kidney through the process involving adherence to tubular cell membranes , followed by internalization of crystals[78].

6.2.MECHANISM OF INDUCTION TECHNIQUE

1. Different chemicals used to induce urolithiasis in experimental animals models by using EG, glycolic acid and ammonium oxalate
2. Animals treated with EG ,it is metabolized in to oxalate particularly in the liver and kidney and its harmlessly excreted
3. Although increase urinary excretion of oxalate (hyperoxaluria) can be toxic largely because of its propensity to crystallize and form the CaOx crystal deposition in kidney.
4. CaOx crystals can block the renal tubules , cellular function, and kill nearby cells (Randall plaques)
5. Ingestion of Ammonium chloride with EG can cause the chronic metabolic acidosis that is accommodated by urinary acidification (Renal tubular acidosis) theses acidosis leads to decrease in excretion of citrate in urine[79]
6. Hypocitaturia caused raised level of ionized calcium and reduces the inhibitory activity against calcium salts . These leads to deposition of CaP crystals in kidney
7. Composed of CaP and RP seem to provide the platform for CaOx crystals to form through heterogenous nucleation and grow in to nephrolithiasis

6.3.Mechanism of Ethylene glycol Toxicity

CaOx crystals alter membrane structure and function , to increase reactive oxygen and to produce mitochondrial dysfunction . This process is likely to be involved in the mechanism of cell death . Accumulation of COM crystals in the kidney is responsible for producing the renal toxicity associated with EG poisoning . The development of a pharmacological approach is to reduce CaOx crystal adherence to tubular cells and its cellular interactions would be valuable as this would decrease the renal toxicity and also in other hyperoxaluric diseases such as primary hyperoxaluria and kidney stone formation[80]

5.5.EXPERIMENTAL DESIGN

Anti-nephrolithiatic activity : Thirty healthy male adult wistar albino rats weighing (150-200gms) were divided in to 5 groups consisting of 6 animals in each group .Ethylene glycol induced urolithiatic model in rats was used to access the effect of ethanolic extract of *Apium graveolens* . The study is designed to find out the effect of ethanolic extracts of *Apium graveolens* on therapeutic usage against ethylene glycol induced nephrolithiasis.

Rats were housed in cages for entire duration of the experiment .The serum of each group was collected for the estimation of bio-chemical parameters.

Urea, Creatinine, SGOT, SGPT, Uric acid are estimated. Therapeutic groups will be sacrificed on 29th day. Right Kidney was examined for the presence of calcium oxalate crystals and stone formation by histological techniques.

Table No:2 Experimental design and treatment plan for the evaluation of nephron lithiatic activity in EEAG

S.NO	STATUS	INDUCTION OF UROLITHIASIS	NUMBER OF ANIMALS	TREATMENT
1	Group I - Normal	---------	6 male Wistar Albino rats	Vehicle
2	Group II – Negative control	0.75%v/v Ethylene glycol in drinking water daily for 28 days orally	6 male Wistar Albino rats	Vehicle
3	Group III – Standard drug treated	0.75%v/v Ethylene glycol in drinking water daily for 28 days orally	6 male Wistar Albino rats	Thiazide (0.9mg/kg) from 15th day to 28th day
4	Group IV -	Ethylene glycol (0.75mg/kg/day, orally)+low dose of drug infusion	6 male Wistar Albino rats	Ethanolic extract of seeds (200 mg/kg) from 15th day to 28th day
5	Group V	Ethylene glycol (0.75mg/kg/day, orally)+ high dose of drug infusion	6 male Wistar Albino rats	Ethanolic extract of seeds (400 mg/kg) from 15th day to 28th day

7.STUDY PLAN

7.1.GENERAL PARAMETERS

1. **Measurement of Body weight**

The weight (in grams) of the animals was noted on the first and Last day of treatment and the percentage change in body weight was calculated.

2. **Measurement of food intake**

Weighed quantity of food was kept each day .Initial and final weight of food was observed for 24 hrs to calculate the food intake values are expressed in grams /24hrs or grams per day

3. **Measurement of water intake**

Initial and final amount of water observed for 24hrs to calculate the water intake values are expressed in grams/24hrs or grams / day

4. **Collection and analysis of urine**

All animals are kept in individual metabolic cages and urine samples of 24hrs were collected on 28^{th}day . Animals had free access to drinking water during the urine collection period. A drop of concentrated HCL was added to the urine befor being stored at 4^0 C. The following urine contents were analysed.

7.2.IN VITRO STUDIES ON ANTINEPHRO-LITHIATIC EFFECT OF ETHANOLIC EXTRACT OF *Apium Graveolens* (EEAG)

To estimate the efficacy of the EEAG in preventing the various stages involved in urolithiasis, the aggregation and nucleation assay were performed with EEAG and compared with the negative control.

Nucleation Assay

Solutions of calcium chloride and sodium oxalate were prepared at a final concentration of 3 mmol/l and 0.5mmol/l respectively, in a buffer containing tris 0.05 mol/l and NaCl 0.15 mol/l at pH 6.5. 950µl of calcium chloride solution was mixed with 100µl of herb extract at different concentrations. Crystallisation was started by adding 950µl of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm. The temperature was maintained at 37^oc. the OD of the solution was monitored at 620 n. The rate of nucleation was estimated by comparing the induction time in the presence of extract with that of the control.

$$\% \, \boldsymbol{Inhibition} = \frac{\boldsymbol{OD\ of\ Sample}}{\boldsymbol{OD\ of\ control}} \times \mathbf{100}$$

Aggregation Assay

Calcium oxalate crystals (COM) were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/l. Both solutions were equilibrated to 60^{o}c in a water bath for 1 hr and then cooled to 37^{o}c overnight. The crystals were harvested by centrifugation and then evaporated at 37^{o}c. COM crystals were used at a final concentration of 0.8mg/l, buffered with Tris 0.05 mol/l and NaCl 0.15 mol/l at pH 6.5. Experiments were conducted at 37^{o}c in the absence and presence of the plant extract. The rate of aggregation was estimated using the formula given below

$$\%\ \boldsymbol{Inhibition} = \mathbf{1} - \frac{\boldsymbol{Turbidity\ of\ sample}}{\boldsymbol{Turbidity\ of\ Control}} \times \mathbf{100}$$

7.3.BIO-CHEMICAL ANALYSIS

At the end of the study on 29^{th}day , the animals were sacrificed under light ether anaesthesia. The rats were sacrificed by decapitation and blood was collected by bleeding of cardiac puncture using centrifuge tubes from all the groups of overnight fasted rats and serum was separated to study the bio-chemical parameters.

Serum analysis

Blood was collected from the rats in each group after sacrificing the animals, and serum was separated by centrifugation at 6000 rpm for 15 min and analyzed for SGOT, SGPT,[81] Serum urea, Uric acid and Creatinine.

Estimation of Urea:

Ammonia and Carbon dioxide (CO2) are produced when urea is hydrolyzed in presence of Urease. The Ammonia produced in the reaction combines with 2- Oxoglutarate and NADH in the presence of Glutamate dehydrogenase (GLDH) to yield glutamate and NAD+ . The NADH/NAD+ reaction produces a unique change in absorbance at 340 nm, which correlates with the concentration of urea nitrogen in the sample.

Estimation of Uric acid:

Uric acid is oxidized to peroxide in presence of an enzyme uricase. The peroxide reacts with ADPS and aminoantipyrine in the presence of an enzyme peroxidase to form a Blue purple quinoneimine dye. The intensity of the Blue purple colour is proportional to the uric acid concentration and is determined photometrically

Estimation of SGPT:

It is an enzymatic method, which measure gultamic pyruvate transaminase in serum according to Reitman and Frankel (1957), and Schmidt and Schmidt, (1963).

Principle:

Alanine amino transfers is measured by monitoring the concentration of pyruive hydrazone formed with 2-4 dinitrophenylhydrazine .

Reaction:

α - oxoglutarate + L-alanine ALT L-glutrate + pyruivate

The absorbance of samples was read against the reagent blank after 5min at

wavelength 546 nm in spectrophotometer.

Estimation of SGOT :

It is an enzymatic method that measure gultamic oxaloacetic transaminase in serum according to Reitman and Frankel (1957), and Schmidt and Schmidt,(1963).

Principle:

Aspartate amino transferase is measured by monitoring the concentration of oxaloacetate hydrozone formed with 2-4 dintrophenyl hydrazine.

Reaction:

α- oxoglutarate +L-aspartate AST L-glutarate + oxaloacetate

The absorbance of samples was read against the reagent blank after 5min at wavelength 546 nm in spectrophotometer and cuvette of 1cm light path.

Haematological studies:

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cells

count (RBC), mean corpuscular volume (MCV), and mean corpuscular

hemoglobin concentration (MCHC), were measured.

Blood samples were collected into dry clean bottles; the anticoagulant was

ethylene diamine tetra acetic acid (EDTA).

Estimation of Creatinine:

Serum creatinine was estimated using Alkaline picrate method.

Reagents :

Picric acid-0.04N

Sodium hydroxide-0.75N

Stock Creatinine (100mg/100ml 0f 0.1N Hcl was made up to 100 ml with distilled water)

Procedure :

0.1ml of the sample was made up to 2ml with water. To this 1ml of picrate and 1ml of sodium hydroxide was added and mixed well and allowed to stand for 15 min at room temperature.

Standard was treated in same manner. After the incubation period the optical density was measured at 560nm against the blank. Creatinine values were expressed in mg/dl.

7.3.Urine Analysis

24 hrs urine sample was collected and calcium, magnesium, urea, uric acid and creatinine were estimated by using standard procedure as follows[82].

Estimation of Urinary Calcium and Magnesium level

1. To a 15ml conical centrifuge tube add 10 ml of well shaken urine , 2 drops of methyl orange , concentrated HCL dropwise , to a red colour and 1ml of 5% ammonium phosphate slowly with shaking.
2. Add 2ml of ammonium hydroxide (28%), mix and let stand for at least 1 hour.
3. Centrifuge , decant and wash the precipitate 3 times with 5 ml of alcohol wash.
4. Dissolve the precipitate with 0.5ml of HCL and wash quantitatively in to a 10ml volumetric flask, bring to volume with distilled water and mix.
5. 5ml of the aliquot from item (4) are taken and 1ml of 2.5% oxalic acid is added, as well as a drop of methyl orange . Sodium acetate solution is then added slowly until the Ph is approximately 4. The mixture is then allowed to stand for 4hrs or more for the complete precipitation of the calcium oxalate .
6. The precipitate of calcium oxalate is then centrifuged and washed twice with 3 ml of 2% ammonium hydroxide . The supernatant and the washings are saved for the magnesium determination

7. The calcium oxalate precipitate is dissolved in 0.5ml of 1:4 Hcl and 5ml of water, 0.5ml of 5% ammonium phosphate and 2ml of ammonium hydroxide are added and mixed to re-precipitate the calcium as the phosphate . After allowing this to stand for 1 hr or more . It is centrifuged and the precipitate washed twice with the alcohol wash. The precipitate may be used for the phosphorus determination ,depending upon the amount of calcium present.
8. To the supernatanat and the washing from the above 0.5ml of ammonium phosphate solution and 2ml of strong ammonia are added and allowed to stand for 1 hr or longer. The precipitate is centrifuged and washed twice with 5 ml of alcohol wash and then redissolved in Hcl. Phosphate analysis is then performed step(5), (3) the magnesium ammonium phosphate from step (9).
9. Spectral range 500-570 nm[83].

Estimation of Urinary Phosphate

A simple qualitative method to determine the presence of phosphate ions in a sample is as follows. A small amount of the sample is acidified with concentrated nitric acid to which a little ammonium molybdate is added.

The presence of phosphate ions is indicated by the formation of a bright yellow precipitate layer of ammonium phosphomolybdate.[105]

Reagents :

a) 10% TCA
b) Ammonium molybdate -2.5% in 5N HCL
c) ANSA -0.5gm of ANSA was dissolved in 5ml of 20% sodium sulphate and then 195 ml of 15% Sodium meta bisulphite was added. Stirred well, filtered and stored in brown bottle.
d) Standard concentration of 8µg/ml was prepared from the stock Potassium dihydrogen phosphate(35.1 mg/100ml)

Procedure :

0.1ml of sample , 4.4 ml of distilled water, 0.5ml of ammonium molybdate, 0.2ml of ANSA

was added and mixed well . The readings were taken after 20mins at 650nm.

Phosphorus values were expressed in mg/dl

7.4.Kidney histopathology

The isolated right kidney was preserved in 10% formalin solution. Sections were cut with 5 µm thickness and using 4 Leica RM-2126 microtome, they were mounted on slides after staining with hematoxylin and eosin. The slides were observed under light microscope to study the kidney architecture and CaOx deposits.

7.5.Statistical Analysis

Results were expressed in terms of mean ± standard error mean. Differences among data were determined using one-way ANOVA test followed by Dunnett's multiple comparison test (GraphPad Software, Inc., version 5, CA, USA.) and $P < 0.05$ was considered statistically significant

8.RESULTS

In this present investigation, an attempt has been made to find out the possible in vivo antiurolithiatic activity of ethanolic extract of *Apium graveolens.*

8.1.Preparation of Extraction

Extraction was done by solvent evaporation method with Soxhlet apparatus. The yield of extraction was found to be 12% w/w.

8.2.PRELIMINARY PHYTO CHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF *APIUM GRAVEOLENS*

The result of preliminary phytochemical analysis of Ethanolic extract of Apium graveolens was shown in the **Table no.3**.

The ethanolic extract of Apium graveolens (EEAG) showed presence of various phytochemical constituents such as Alkaloids, Flavanoids, Glycoside, tannins, Phenols and steroids.

Tableno : 3 Phytochemical screening of Apium Graveoelens

Phytochemical studies		
TESTS		**OBSERVATION**
Alkaloids		
1.Drogendroff's test		+
2. Wagner's test		+
3.mayer's test		+
Flavanoids		+
Saponins		Traces
Carbohydrates		
1.	Fehlings test	+
2.	Benedicts test	+
3.	Mollischs test	+
Phenols		
1.	Ferric chloride test	+
2.	Lead acetate test	+
3.	Liebermanns test	+
Steroid		

1.	Libermanns-Burchards test	+
2.	Salkowsi reaction	+
Glycosides		+
Tannins		
1.	Ferric chloride test	+
2.	Lead acetate test	+
Terpenoids		-
Sterols		Traces
	(+)…….. Positive	**(-)..... Negative**

8.3. *IN VITRO* RESULTS

Effect of EEAG on Nucleation Assay

Table No : 4 – Invitro nucleation assay results

Concentration (mg)	**Absorbance (nm)**
Control	0.176
0.5	0.127
1.0	0.108

Effect of EEAG on Aggregation Assay

Table No:5 – Invitro aggregation assay results

Concentration (mg)	**% Inhibition of aggregation**	
	At 90 min interval	**At 180 min interval**
Control	0	0
0.5	1.25	45.11
1.0	5.11	17.75

8.4.ANIMAL STUDIES

Results of Acute oral toxicity Study:

The Acute Oral Toxicity study was done according to the OECD Guidelines 423 { Acute toxic class method }. A single dose of 2000 mg/kg b.w / p.o EEAG was administered to three rats and observed for 3 days. There was no considerable change in body weight before and after treatment and no sign of toxicity was observed. No change was observed from first set of experiment. Results are showed in Table

Acute Oral Toxicity studies of EEAG (OECD 423 Guidelines)

Table No :6 – Acute oral toxicity Effect on Ethanolic extraction of *Apium graveolens seeds*

S.no	Treatment group	Dose	Weight of the animal in grams		Signs of toxicity	Onset of toxicity	Reversible or Irreversible	Duration
			Before test	After test				
1	EEAG	2g/kg	210	220	No signs of toxicity	Nil	Nil	14 Days
2	EEAG	2g/kg	210	230	No signs of toxicity	Nil	Nil	14 Days
3	EEAG	2g/kg	250	260	No signs of toxicity	Nil	Nil	14 Days

8.5.Antinephrolithiatic activity of EEAG

GENERAL PARAMETERS :

EFFECT OF EEAG ON BODY WEIGHT

A Significant decrease in body weight ($p<0.05$) was observed in negative control when compared to the control group. The group (3,4,5) treated with EEAG showed significant (**$p<0.01$).(*$p<0.05$) shows increase in body weight when compare to negative control.

The results were shown in the Table no.7 and Figure no.9.

EFFECT OF EEAG ON FOOD INTAKE

A Significant (**$p<0.01$,***$p<0.001$) decrease in food intake was seen at negative control than the control group. The group (3,4,5) treated with EEAG shows significant (**$p<0.01$)(***$p<0.001$) there is the increase in food intake when compared to the negative control.

The results were shown in the Table no.8 and Figure no.10.

EFFECT OF EEAG ON WATER INTAKE

A Significant (*$p<0.01$,**$p<0.001$) decrease in water intake was seen at negative control than the control group. The group (3,4,5) treated with EEAG shows significance (*$P<0.05$,**$P<0.01$) increase in intake of food when compared to the negative control.

The results were shown in the Table no.9 and Figure no.11.

EFFECT OF EEAG ON URINE VOLUME

A Significant (*$p<0.05$,**$P<0.01$) decrease in urine volume is observed in negative control when compared to the control. The group treated with the EEAG shows significance (*$p<0.01$) increase in the urine volume when compared to the negative control.

The results were shown in the Table no.10 and Figure no.12.

Effect of EEAG on Body weight in grams:

TABLE: 7-Effect of EEAG on Body weight in grams

GROUP	BODY WEIGHT	
	14th day	28th day
Control	192.000 ±4.063	208.555 ±3.333
Negative Control	175.663 ±6.666*a	182.666 ± 6.009*a
Standard	205.555 ±7.719*b	226.333± 8.279*b
EEAG 200mg/kg	195.555 ±18.559**b	200.666± 4.409**b
EEAG 400mg/kg	185.000 ±4.582*b	216.333± 2.279*b

Values are expressed as Mean ± SEM, n=6

Comparison : a -Group I vs Group II

b- Group II vs Group III, IV& V; ^{NS} Non significant;

*P<0.05, **P<0.01;***P<0.001

One way ANOVA followed by Dunnet's "t" Test

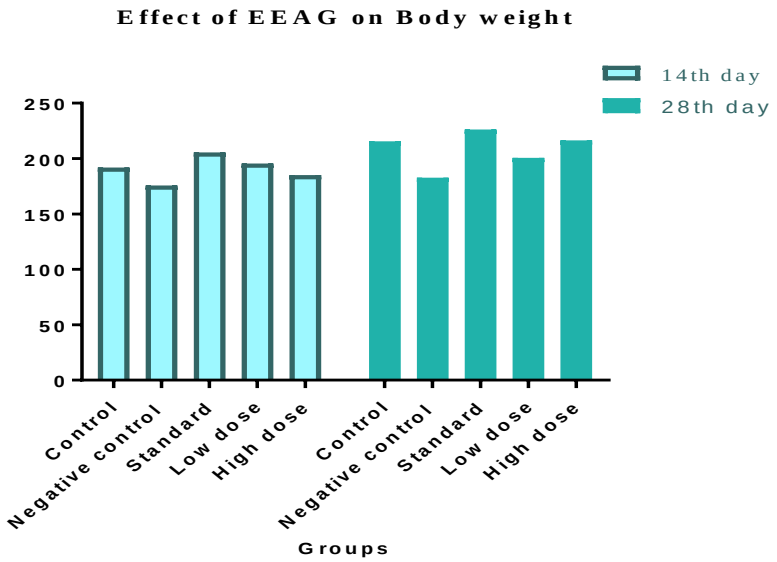


Figure No: 9 :Effect of EEAG on Body weight in grams

Effect of EEAG on Food Intake in grams:

TABLE :8 Effect of EEAG on Food intake gm/ 24 hr

GROUP	FOOD INTAKE	
	14th day	28th day
Control	67.500± 0.907	75.166± 0.333
Negative Control	48.900± 3.450**a	42.300 ± 3.963***a
Standard	61.696± 3.758*b	74.633± 3.35*b
EEAG 200mg/kg	52.677± 2.628**b	68.100± 2.79**b
EEAG 400mg/kg	54.309± 3.081**b	74.300 ± 2.63*b

Values are expressed as Mean ± SEM, n=6

Comparison : a -Group I vs Group II

b- Group II vs Group III, IV& V; ^{NS} Non significant;

*P<0.05, **P<0.01;***P<0.001

One way ANOVA followed by Dunnet's "t" Test

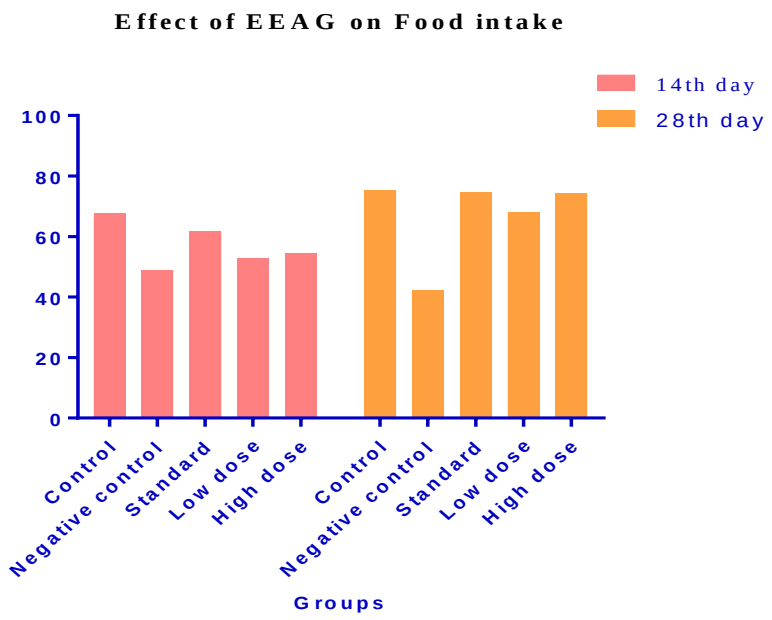


Figure No : 10Effect of EEAG on Food intake gm/ 24 hr

Effect of EEAG on Water intake in ml/24hr:

Table : 9 Effect of EEAG on Water intake in ml/24hr

GROUP	WATER INTAKE	
	14th day	28th day
Control	35.600±1.233	36.377±1.005
Negative Control	24.533± 1.426**a	13.433± 0.520***a
Standard	32.100± 0.702*b	33.333± 0.440**b
EEAG 200mg/kg	30.733± 0.959**b	32.533± 0.688*b
EEAG 400mg/kg	26.655± 0.866*b	31.766± 0.176*b

Values are expressed as Mean ± SEM, n=6

Comparison : a -Group I vs Group II
b- Group II vs Group III, IV& V;^{NS} Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet's "t" Test

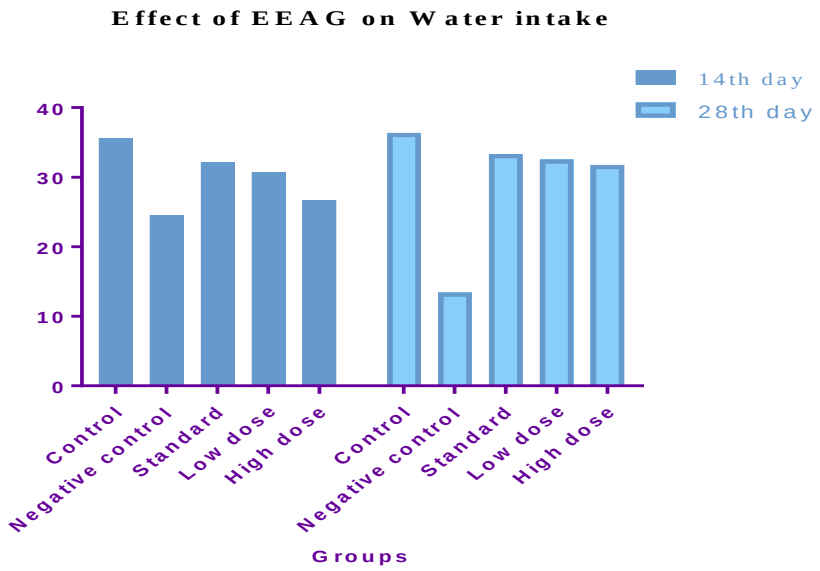


Figure No : 11 Effect of EEAG on Water intake in ml/24hr

Effect of EEAG on Urine output in ml/24hr

Table :10 Effect of EEAG on Urine output in ml/24hr

GROUP	URINE OUTPUT	
	14th day	28th day
Control	10.55± 0.72	9.67± 0.56
Negative Control	7.55± 0.23**a	5.25± 0.35**a
Standard	10.88± 0.5***b	9.12± 0.62***b
EEAG 200mg/kg	8.44± 0.2*b	6.25± 0.4*b
EEAG 400mg/kg	9.22± 0.41**b	7.95± 0.8**b

Values are expressed as Mean ± SEM, n=6
Comparison : a -Group I vs Group II
b- Group II vs Group III, IV& V; ^{NS} Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet's "t" Test

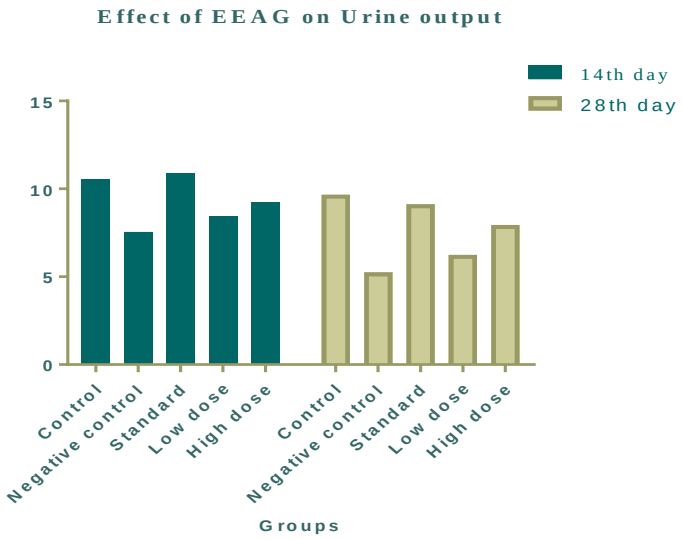


Figure No : 12 Effect of EEAG on Urine output in ml/24hr

BIO-CHEMICAL PARAMETERS

EFFECT OF EEAG ON CREATININE

There was significant ($p<0.01$) increase in serum creatinine in ethylene glycol induced group when compared to control group. There was significant ($p<0.01$) decrease in creatinine in thiazide treated group when compared to control group. There was significant ($p<0.05$) decrease in serum creatinine in EERI treated group at a dose of 200mg/kg/po when compared to control group. There was significant ($p<0.001$) decrease in creatinine in EERI treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.01$) decrease in serum creatinine in thiazide treated rats when compared ethylene glycol induced pump. The EERI at a dose of 200mg/kg/p.o showed a significant ($p<0.01$) decrease in serum creatinine when compared to ethylene glycol induced group. The EEAG at a dose of 400 mg/kg/p.o showed a significant ($p<0.001$) decrease in creatinine when compared to ethylene glycol induced group.

The results were shown in the Table no11.and Figure no.13.

EFFECT OF EEAG ON URIC ACID

There was significant ($p<0.01$) increase in uric acid in ethylene glycol induced group when compared to control group. There was significant ($p<0.05$) decrease in uric acid in thiazide treated group when compared to control group. There was significant ($p<0.01$) decrease in thiazide in EEAG treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.05$) decrease in uric acid in EEAG treated group at a dose of

400mg/kg/p.o when compared to control group.

There was a significant ($p<0.01$) decrease in Uric acid in thiazide treated rats when compared ethylene glycol induced pump. The EEAG at a dose of 200mg/kg/p.o showed a significant ($p<0.01$) decrease in serum creatinine when compared to ethylene glycol induced group. The EEAG at a dose of 400 mg/kg/p.o showed a significant ($p<0.05$) decrease in Uric acid when compared to ethylene glycol induced group.

EFFECT OF EERI ON SGOT

There was significant ($p<0.01$) increase in serum in ethylene glycol induced group when compared to control group. There was significant ($p<0.05$) decrease in serum SGOT in thiazide treated group when compared to control group. There was significant ($p<0.01$) decrease in serum SGOT in EEAG treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.05$) decrease in serum SGOT in EEAG treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.01$) decrease in serum SGOT level in thiazide treated rats when compared ethylene glycol induced pump. The EEAG at a dose of 200mg/kg/p.o showed a significant ($p<0.05$) decrease in serum SGOT level when compared to ethylene glycol induced group. The EEAG at a dose of 400 mg/kg/p.o showed a significant ($p<0.01$) decrease in serum SGOT level when compared to ethylene glycol induced group.

The results were shown in the Table no.11 and Figure no.14.

EFFECT of EERI on SGPT

There was significant ($p<0.001$) increase in serum glutamic pyruvate transaminase level in ethylene glycol induced rats when compared to control group. There was significant ($p<0.05$) decrease in SGPT in thiazide treated group when compared to control group.

There was significant ($p<0.05$) decrease in serum SGPT in EEAG treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.001$) decrease in serum SGPT in EEAG treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.001$) decrease in serum SGPT level in thiazide treated rats when compared ethylene glycol induced group. The EEAG at a dose of 200mg/kg/p.o showed a significant ($p<0.05$) decrease in serum SGOT level when compared to ethylene glycol induced group. The EEAG at a dose of 400 mg/kg/p.o showed a significant ($p<0.001$) decrease in serum SGOT level when compared to ethylene glycol induced group.

The results were shown in the Table no 12.and Figure no.15.

EFFECT of EERI on UREA

There was significant ($p<0.001$) increase in Urea level in ethylene glycol induced rats when compared to control group. There was significant ($p<0.001$) decrease in Urea in thiazide treated group when compared to control group.

There was significant ($p<0.05$) decrease in serum Urea in EEAG treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.05$) decrease in serum Urea in EEAG treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.001$) decrease in serum Urea level in thiazide treated rats when compared ethylene glycol induced group. The EEAG at a dose of 200mg/kg/p.o showed a significant ($p<0.05$) decrease in serum Urea level when compared to ethylene glycol induced group. The EEAG at a dose of 400 mg/kg/p.o showed a significant ($p<0.05$) decrease in serum Urea level when compared to ethylene glycol induced group

The results were shown in the Table no 11.and Figure no.14.

TABULAR COLUMN :

Table :11 Effect of EEAG on Serum parameters – Creatinine, Urea, Uric acid

GROUP	SERUM PARAMETERS		
	Creatinine	Urea	Uric acid
Control	0.85± 0.115	18.22±0.51	0.83± 0.64
Negative Control	1.000± 0.04**a	26.99±0.02***a	2.89± 0.63**a
Standard	0.800± 0.005**b	20.26±0.16***b	1.17± 0.71*b
EEAG 200mg/kg	0.910± 0.011*b	25.37± 0.18*b	1.10± 1.02**b
EEAG 400mg/kg	0.752± 0.051***b	27.35± 0.22*b	1.76± 0.56*b

Values are expressed as Mean ± SEM, n=6
Comparison : a -Group I vs Group II
b- Group II vs Group III, IV& V;^{NS} Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet's "t" Test

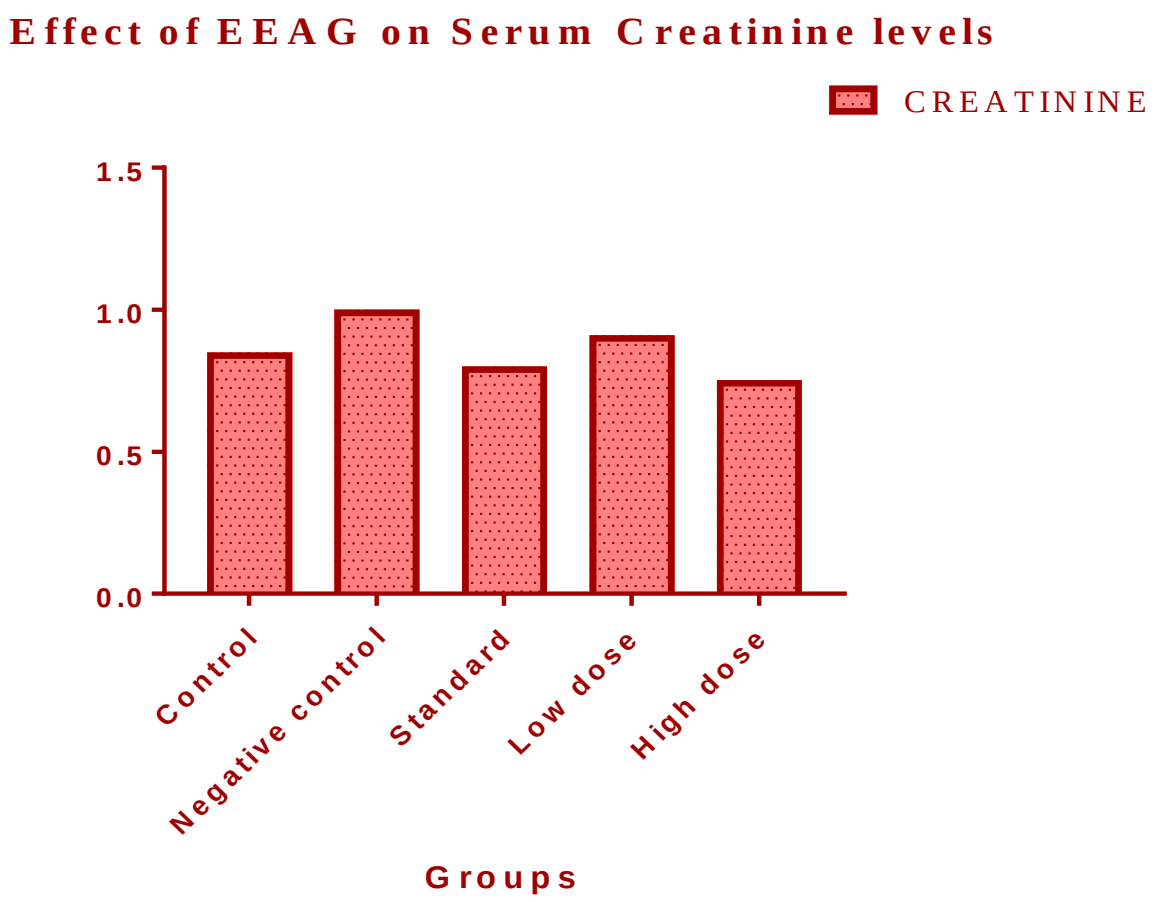


Figure No :13Effect of EEAG on Serum parameters – Creatinine

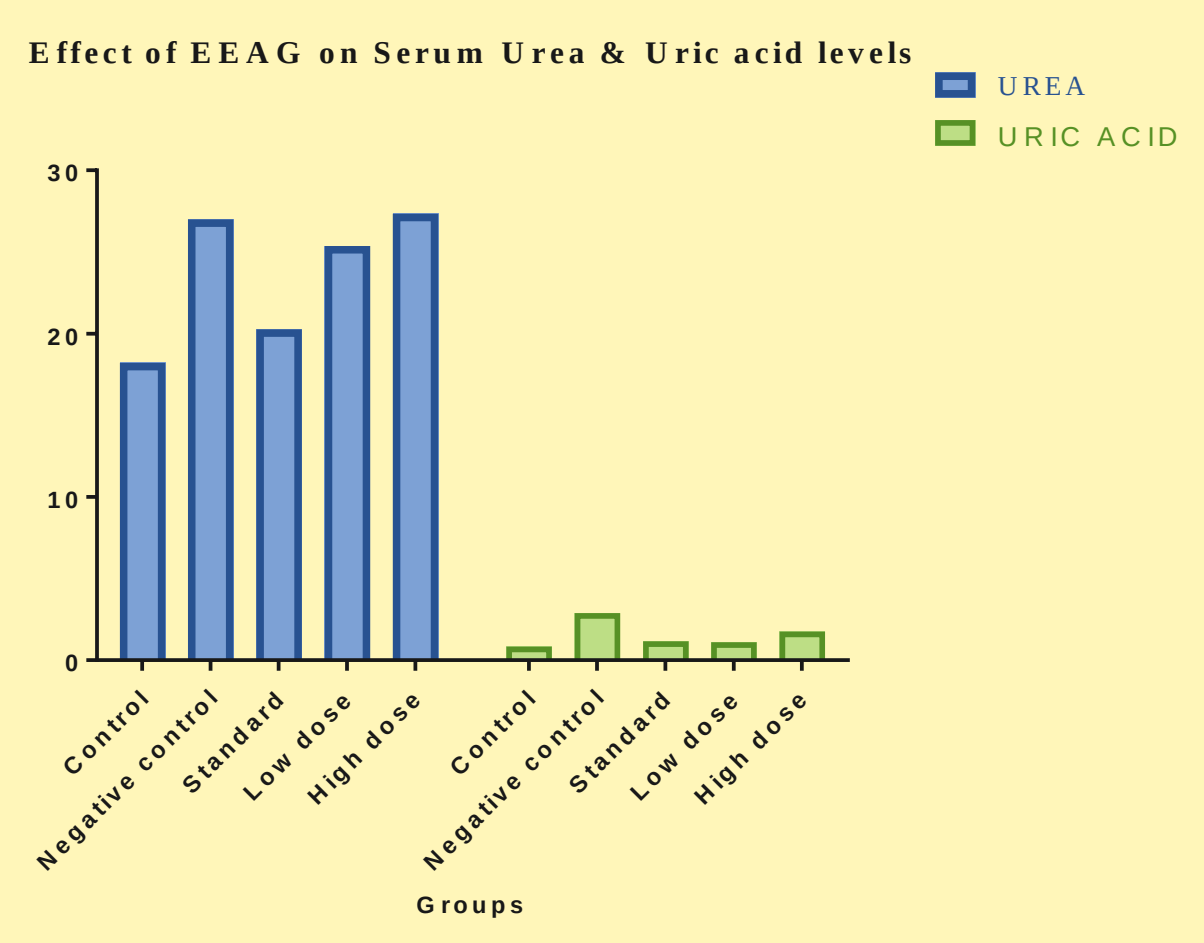


Figure No.14 Effect of EEAG on Serum parameters – Creatinine, Urea, Uric acid

Table :12 Effect of EEAG on Serum parameters – SGOT & SGPT

GROUP	SERUM PARAMETERS	
	SGPT	SGOT
Control	35.06± 0.12	72.47± 0.17
Negative Control	53.47± 0.01**a	90.99± 0.01***a
Standard	37.80± 0.03*b	74.77± 0.19*b
EEAG 200mg/kg	35.56± 0.72**b	80.44± 0.22*b
EEAG 400mg/kg	40.98± 0.11*b	73.99± 0.01***b

Values are expressed as Mean ± SEM, n=6
Comparison : a -Group I vs Group II
b- Group II vs Group III, IV& V; ^{NS} Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet's "t" Test

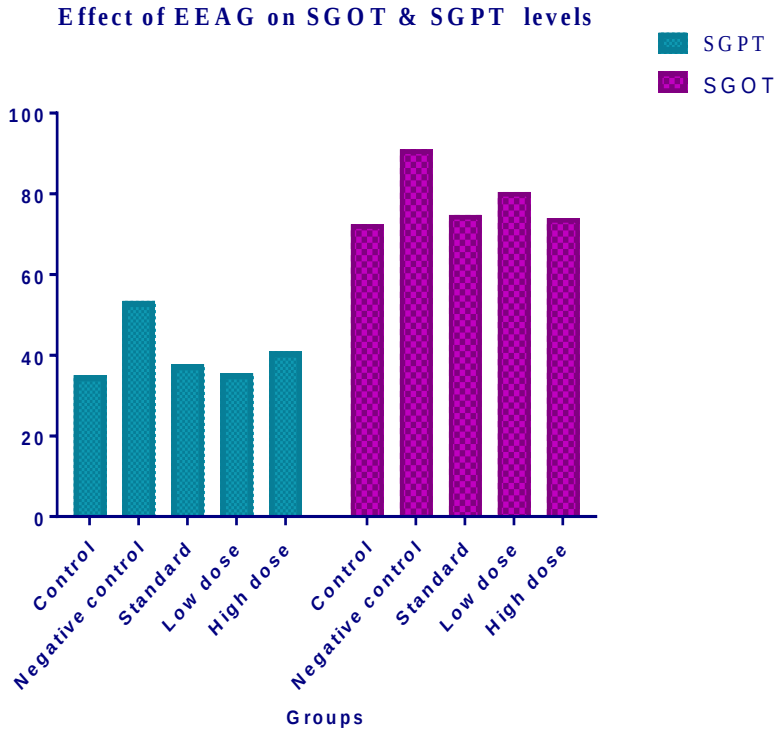


Figure No 15 Effect of EEAG on Serum parameters – SGOT & SGPT

URINE PARAMETERS

EFFECT OF EEAG ON URINARY CALCIUM

There was significant ($p<0.01$) increase in urinary calcium level in ethylene glycol induced rats when compared to control rats. There was significant ($p<0.05$) decrease in urinary calcium in thiazide treated group when compared to control group. There was significant ($p<0.01$) decrease in urinary calcium in EERI treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.05$) decrease in urinary calcium in EERI treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.01$) decrease in urinary calcium in thiazide treated group when compared ethylene glycol induced group. The EEAG at a dose of 200mg/kg/p.o showed a significant ($p<0.01$) decrease in urinary calcium when compared to ethylene glycol induced group. The EERI at a dose of 400 mg/kg/p.o showed a significant ($p<0.05$) decrease in urinary calcium when compared to ethylene glycol induced group.

EFFECT OF EEAG ON URINARY MAGNESIUM

There was significant ($p<0.01$) increase in urinary magnesium level in ethylene glycol induced rats when compared to control rats. There was significant ($p<0.001$) increase in magnesium in thiazide treated group when compared to control group. There was significant ($p<0.05$) increase in magnesium in EERI treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant ($p<0.05$) increase in magnesium in EERI treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant ($p<0.01$) increase in urinary magnesium in thiazide treated group when compared ethylene glycol induced group. The EERI at a dose of 200mg/kg/p.o showed a significant ($p<0.05$) increase in urinary magnesium when compared to ethylene glycol induced group. The EERI at a dose of 400 mg/kg/p.o showed a significant ($p<0.05$) increase in urinary magnesium when compared to ethylene glycol induced group.

The results were shown in the Table no.4 and Figure no.9.

EFFECT OF EEAG ON URINARY PHOSPHATE

A Significant (**$p<0.01$) increase in phosphate is observed at ethylene glycol induces rats when compared to the control group. There was significant ($p<0.01$) decrease in urinary phosphate in thiazide treated group when compared to control group. There was significant

(p<0.01) decrease in urinary phosphate in EEAG treated group at a dose of 200mg/kg/p.0 when compared to control group. There was significant (p<0.05) decrease in urinary phosphate in EEAG treated group at a dose of 400mg/kg/p.o when compared to control group.

There was a significant (p<0.01) decrease in urinary phosphate in treated group when compared ethylene glycol induced group. The EERI at a dose of 200mg/kg/p.o showed a significant (p<0.01) decrease in urinary phosphate when compared to ethylene glycol induced group. The EEAG at a dose of 400 mg/kg/p.o showed a significant (p<0.05) decrease in urinary phosphate when compared to ethylene glycol induced group.

Table :13 Effect of EEAG on Urinary parameters -Calcium , Magnesium and Phosphate.

GROUP	URINE PARAMETERS		
	Calcium	**Magnesium**	**Phosphate**
Control	2.733± 0.520	0.20± 0.13	4.807± 0.606
Negative Control	5.800± 0.603**a	0.36± 1.04**a	8.520± 0.952**a
Standard	2.613± 0.58*b	0.22± 0.69***b	4.607± 0.555**b
EEAG 200mg/kg	2.544± 0.317**b	0.27± 0.66*b	4.033± 0.543**b
EEAG 400mg/kg	2.722± 0.548*b	0.28± 0.78*b	5.001± 0.544*b

Values are expressed as Mean ± SEM, n=6
Comparison : a -Group I vs Group II
b- Group II vs Group III, IV& V; ^{NS} Non significant;
*P<0.05, **P<0.01;***P<0.001
One way ANOVA followed by Dunnet's "t" Test

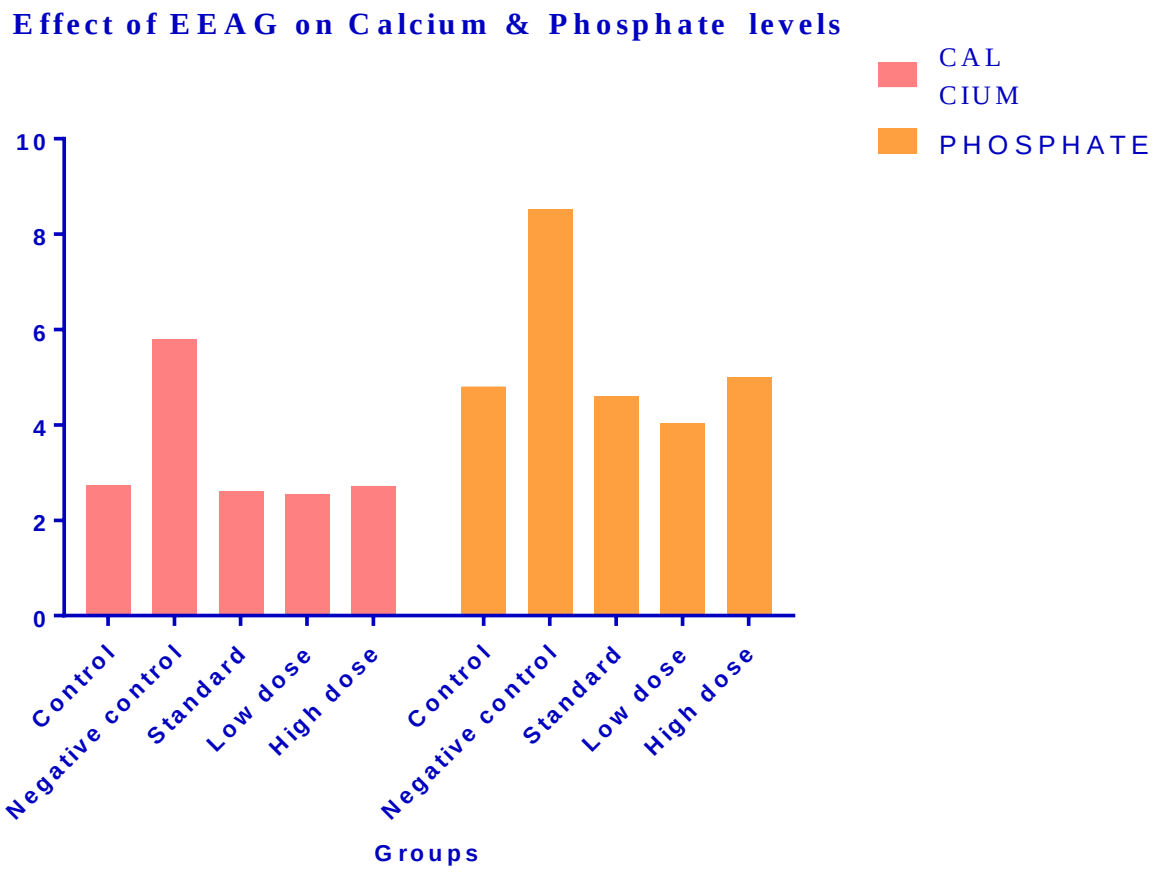
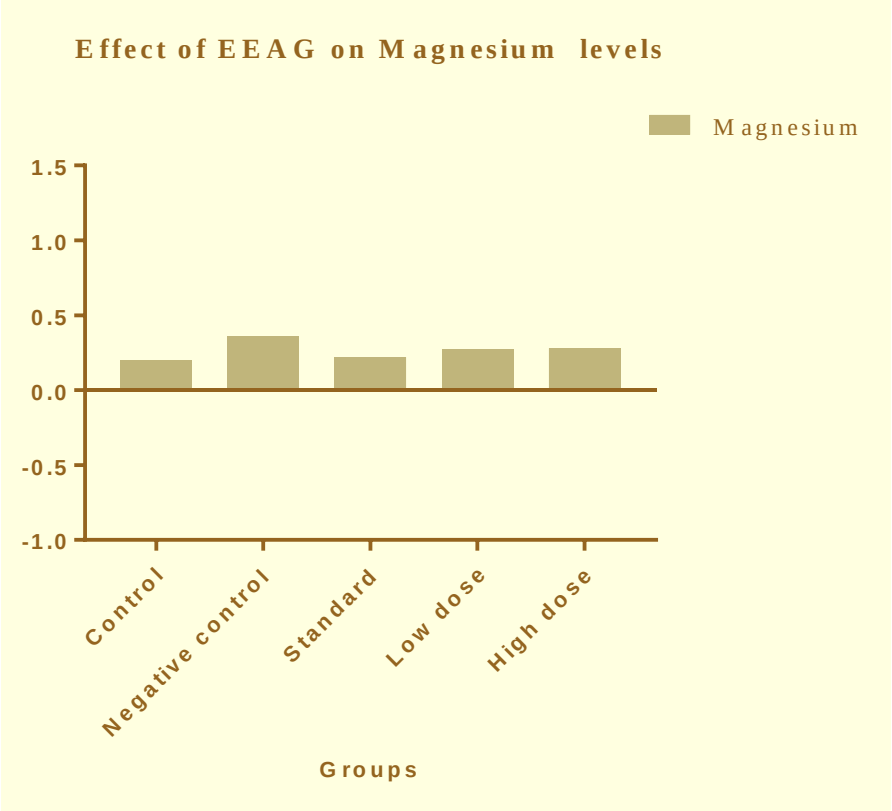


Figure No : 16 Effect of EEAG on Urinary parameters -Calcium and Phosphate.



No.17 Effect of EEAG on Urinary parameters - Magnesium

HISTOPATHOLOGY RESULTS

Effect of EEAG on Renal stones

a) GROUP-I Control

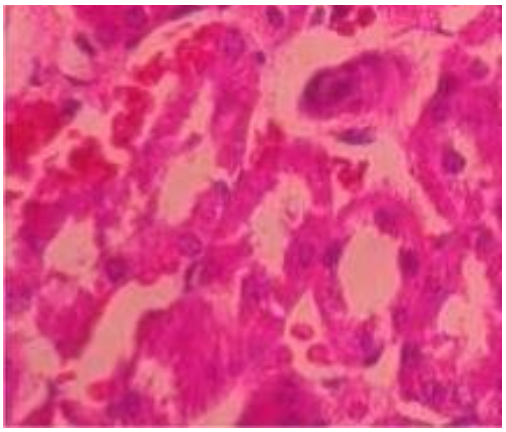


Figure 18

Effect of EEAG on Renal Stones

b) GROUP-II Negative Control

Figure 19

Effect of EEAG on Renal Stones

c) GROUP-III Standard

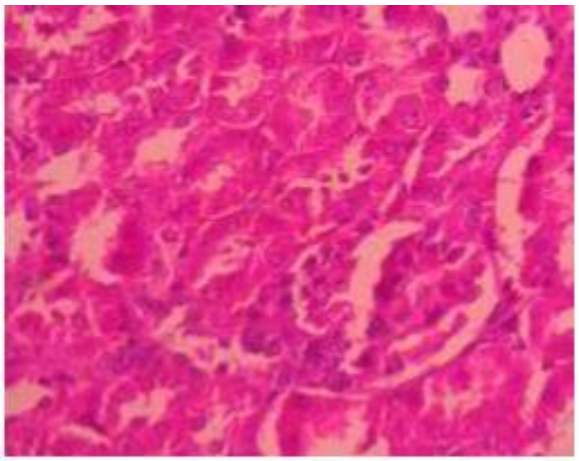


Figure 20

a) GROUP-IV High dose

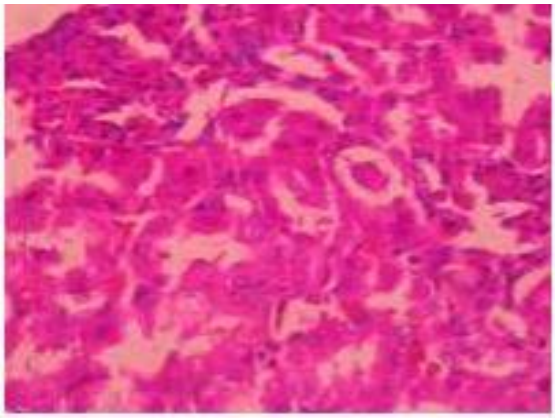


Figure 21

d) GROUP-IV High dose

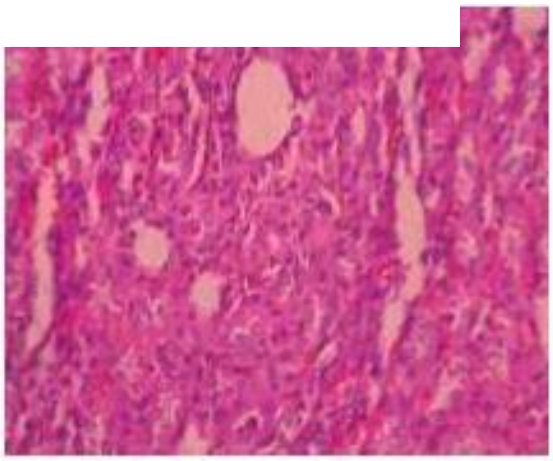


Figure 22

9.DISCUSSION

Estimated life time risk of Urolithiasis in Asia 2%-5%, 8%-15% in Europe and America and around 20% in middle east. It is associated with a high risk of reoccurrence which is around 10%-23% per year 50% in 10 years and 75% in 20 years.

Stones may occur in any part of the urinary system like kidney, ureter, bladder, and one of the most painful diseases. Urinary lithiasis is due to imbalance between inhibitors and

promoters in the kidneys. Urinary super saturation is the driving force behind crystal formation in the kidneys. Since formation of crystalline particles must obviously start from super saturation. Super saturation is undoubtedly essential for stone formation. The initial step in the transformation from a liquid to a solid phase in a supersaturated solution is called nucleation. This process begins with the coalescence of stone salts in solution into loose clusters that may increase in size by addition of new components or clusters. Once a crystal nucleus has achieved a critical size and relative supersaturation remains above 1, overall free energy is decreased by adding new crystal components to the nucleus. This process is called crystal growth. Aggregation is a process in which the salts in the solution stick together to form larger particles. Some researchers have Crystallization is caused by the condition of urinary supersaturation. Then, the crystals that have formed attach to renal tubular epithelial cells and are taken into them; crystal aggregation is the most important step in stone formation.

Apium graveolens seeds are commonly usedin the nativesystem of medicine. Various parts of the plant like leaves and roots are medicinally important.

In order to investigate the medicinal use of *Apium graveolens* in urolithiasis, we evaluated crude extract for its antiurolithic activity using different *in vitro* assays and *in vivo* rat model of urolithiasis.

Calcification is a multifactorial phenomenon[96] developing as a result of a cascade of events initiated by supersaturation, including crystal nucleation, growth, aggregation and retention[97] Crystal inhibitors thiazide like have been shown to decrease the saturation of CaOx and inhibit crystal nucleation, growth and aggregation, while reduced crystallization in urine of stone forming inn rats [98]. Interference with crystal growth and aggregation therefore seems a possible therapeutic strategy for the prevention of recurrent stone disease. Preliminary Phytochemical analysis of ethanolic extract of Apium graveolens had showed the presence of Phytoconstituents like alkaloids, flavonoids, tannins, saponins and Cardiac glycosides. Flavanoids and alkaloids are widely distributed in the plant which have the property to cure urolithiasis. Due to this reason the plant has chosen to this study. This shows that the EEAG may contain substances that inhibit CaOx crystal aggregations and thus preventing a critical step in urinary stone formation, as larger particles are less likely to pass spontaneously in urinary tract. If the extract keeps CaOx particles dispersed in solution they can be easily eliminated [99].

Decrease in body weight in ethylene glycol and ammonium chloride treated group was observed. There is no change in body weight in Thiazide treated group and there was a significant Increase in body weight of EEAG treated group given at both the doses of 200 mg/kg and 400 mg/kg.

On examining the renal function tests of ethylene glycol and ammonium chloride induced animals, the excretion of uric acid, urea, creatinine and calcium has significantly increased and magnesium has significantly decreased with that of the control group. After treatment with the ethanolic extract of *Apium graveolens* (200 mg/kg and 400 mg/kg) the excretion of uric acid, urea, creatinine and calcium has significantly decreased and the excretion of magnesium has significantly increased. Although the low dose was more potent than the high dose when compared with thiazide treated group, which is a standard.

Ethanolic extract of *Apium graveolens* has shown promising in vitro efficacy on urolithiasis, we have observed increase in the absorbance indicating the inhibition of Nucleation and Aggregation of calcium oxalate in in vitro studies.

For the *in vivo* antiurolithic effect of EEAG, 0.75% ethylene glycol (EG) and 1% ammonium chloride (AC)-induced hyperoxaluric rat model of urolithiasis was used. Since the stone inducing treatment, Ethylene glycol (EG), was given orally, therefore, the extract was given i.p. in order to prevent any potential interaction of EG with plant constituents inside gut, interfering with absorption of either of the two. Administration of EG and AC resulted in the increased CaOx crystalluria, with larger crystals due to hyperoxaluria, increase in water intake and urine output, which might be due to the renal impairment [100] as evident by increase in serum creatinine, blood urea in lithiatic group as compared to normal[101,102]

There was hypertrophy and extensive CaOx crystal deposition in kidneys of untreated rats. The renal tubules were markedly dilated, which might be due to the obstruction in distal renal tubular flow by large crystals [100]Several *in vivo* and *in vitro* studies have demonstrated that hyperoxaluria, a major risk factor for calcium oxalate nephrolithiasis, results in greater production of superoxide and hydroxyl free radicals, leading to antioxidant imbalance, cell membrane rupture and cell death [103]which leads to CaOx crystal adherence and retention in renal tubules [104.] Thus, it can be speculated that the inhibitory effect of the plant extract on CaOx crystal deposition in renal tubules is possibly caused by its antioxidant activity. The plant is considered relatively safer, as it has been used in different herbal preparations and supplements , is used in humans for ailment of diabetic and urinary disorders, which has also

undergone clinical trials for several studies, with no reported side effect, for anti-inflammatory and noci captive properties

Thus, these data suggest that the effect of EEAG in urolithiasis is mediated its effect through multiple pathways including inhibition of the CaOx crystal aggregation, which provide a step forward for designing further studies on EEAG to establish its safety and efficacy for clinical use.

10.SUMMARY AND CONCLUSION

Urolithiasis is one of the most painful urological disorder when compared to the other painful disorders. Kidney stone disease has afflicted human kind since antiquity and can persist, with serious medical consequences, throughout a patient's life time. In addition, the incidence of kidney stones has been increased in most societies in the last five decades, especially in association with economic development. In spite of tremendous advances in the field of medicine, there is truly no satisfactory drug for the treatment of nephrolithiasis.

Recently, there is increasing evidence that many healthy natural food and medicinal herbal and supplements have the potential to become valuable complementary therapy in the treatment of various renal disorders and in the protection against nephrotoxicity.

The plant *Apium graveolens* is traditionally used for in different disorders such as anti-inflammatory activity, anticancer, antidiabetic, , hepatoprotective activity, etc.. An attempt was made to study urolithiatic activity in ethylene glycol and ammonium chloride induced method.

The preliminary phytochemical analysis of EEAG showed the presence of alkaloids, carbohydrates, cardiac glycoside, sterols & saponins, tannins, flavonoids are used against urolithiasis.

The anti-urolithiasis activity of *Apium graveolens seeds* is may be due to the presence of flavonoids and tanins.

Animals were divided in to 5 groups . Group I-Control, Group II-Negative control, Group III-Treated with standard drug, Group IV-Treated with Lower dose , Group-IV-Treated with higher dose. Data comparison was made between Group I – Group V .Urolithiasis was induced by feeding Ethylene glycol with ammonium chloride for 28 days. Statistical significance was done by ANOVA , followed by Dunnett's multiple comparison test

- CaOx and CaP deposition in the kidneys of EG and ammonium chloride fed animals . Treatment with EEAG (200/400 mg/kg) for 14 days successfully prevented the elevation of deposition of CaOx , phosphate in kidney when compared to the standard drug.
- Significant decrease in Body weight, Feed intake, water intake observed in EG fed animals . Treatment with EEAG (200/400 mg/kg) for 14 days successfully increases the Body weight, water intake and food intake , when compared to the standard drug.

- Increased urinary excretion of urinary calcium , oxalate, and phosphate are observed in EG and ammonium chloride fed animals . Treatment with EEAG (200/400 mg/kg) for 14 days successfully decreased the excretion of oxalate, calcium, phosphate when compared to the standard drug.
- Serum creatinine , uric acid, Urea increased in EG and ammonium chloride fed animals due to decreased GFR. Treatment with EEAG (200/400 mg/kg) for 14 days successfully decreases the serum creatinine, uric acid, Urea when compared to standard drug.
- Histo pathological findings of the EG fed animals are distended tubules, dilation and deposition of crystals. The histopathology of kidney was brought to normal in EEAG treated animals when compared to the standard drug.

The present study indicates that the administration of EEAG to rats in ethylene glycol and ammonium chloride induced urolithiasis reduces the growth and development of kidney stones by reducing the stone forming constituents by increasing the GFR . Accordingly, it can be concluded that the supplementation of *Apium graveoelns* has a beneficial effect on urolithiasis .

Further studies are needed to identify the molecular mechanism of *Apium graveoelns* and the structural elucidation of phytoconstituents responsible for antiurolithiasis

11.BIBLIOGRAPHY

1. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacol. 2000;71:23–43. [PubMed]
2. Khan MN, Nizami SS, Khan MA, Ahmed Z. New saponins from Mangifera Indica. J Nat Prod. 1993;56:767–70. [PubMed]
3. Shankarnarayanan D, Gopalakrishman C, Kameswaran L, Arumugum S. The effect of mangostin, mangostin-3, 6-di-O-glucoside and Mangiferin in carbon tetrachloride liver injury. Mediscope. 1979;22:65.
4. Khan MA, Khan MN. Alkyl gallates of flowers of Mangifera Indica. Fitoterapia. 1989;60:284.
5. Ross IA. Vol. 1. New Jersey Totowa: Human Press; 1999. Medicinal plants of the world; pp. 199–200.
6. Shibahara A, Yamamoto K, Shinkai K, Nakayama T, Kajimoto G. Cis-9, cis-15-octadecadienoic acid:a novel fatty acid found in higher plants. Biochimi Biophy Acta. 1993;1170:245–52. [PubMed]
7. Coe FL, Keck J, Norton ER. The natural history of calcium urolithiasis. Jama. 1977;238:1519–1523.[PubMed
8. Pearle MS, Calhoun EA, Curhan GC, Urologic diseases in America project: Urolithiasis, J Urol 2005; 173: 848–57.
9. 9.Hughes P, Kidney stones epidemiology. Nephrol 2007; 12: 26–30.
10. Dawson PA, Russell CS, Lee S, McLeay SC, van Dongen JM, Cowley DM, Clarke LA, Markovich D. Urolithiasis and hepatotoxicity are linked to the anion transporter Sat1 in mice, J Clin Invest 2010; 120(3): 706–12.
11. Saigal CS, Joyce G, Timilsina AR, Direct and indirect costs of nephrolithiasis in an employed population: Opportunity for disease management? Kidney Int 2005; 68: 1808–14.
12. Choubey A, Choubey A, Jain P, Iyer D, Patil UK, Assessment of Ceiba pentandra on calcium oxalate urolithiasis in rats. Der Pharma Chemica 2010; 2(6): 144–56.
13. Macneil F, Bariol S; Urinary stone disease - assessment and management. Aust Fam Physician. 2011 Oct 40(10):772-5.
14. Esparza Martin N, Garcia Nieto V; Hypouricemia and tubular transport of uric acid. Nefrologia. 2011 31(1):44-50. doi: 10.3265/Nefrologia.pre2010.Oct.10588.

15. Jeong JY, Doo SW, Yang WJ, et al; Differences in Urinary Stone Composition according to Body Habitus. Korean J Urol. 2011 Sep 52(9):622-5. Epub 2011 Sep 28.
16. NKF (National Kidney Federation)
17. Worcester EM, Coe FL; Clinical practice. Calcium kidney stones. N Engl J Med. 2010 Sep 2 363(10):954-63.
18. Renal or ureteric colic - acute; NICE CKS, April 2015 (UK access only)
19. Wood HM, Shoskes DA; The role of nanobacteria in urologic disease. World J Urol. 2006 Feb 24(1):51-4. Epub 2006 Jan 10.
20. Shiekh FA, Khullar M, Singh SK; Lithogenesis: induction of renal calcifications by nanobacteria. Urol Res. 2006 Feb 34(1):53-7. Epub 2006 Jan 20.
21. Evan A, Lingeman J, Coe FL, et al; Randall's plaque: pathogenesis and role in calcium oxalate nephrolithiasis. Kidney Int. 2006 Apr 69(8):1313-8.
22. Schwartz BF, Stoller ML; The vesical calculus. Urol Clin North Am. 2000 May 27(2):333-46.
23. Torricelli FC, Mazzucchi E, Danilovic A, et al; Surgical management of bladder stones: literature review. Rev Col Bras Cir. 2013 May-Jun 40(3):227-33.
24. Stav K, Dwyer PL; Urinary bladder stones in women. Obstet Gynecol Surv. 2012 Nov 67(11):715-25. doi: 10.1097/OGX.0b013e3182735720.
25. Garcia Lopez FJ, Quereda C; Melamine toxicity: one more culprit in calcium kidney lithiasis. Kidney Int. 2011 Oct 80(7):694-6. doi: 10.1038/ki.2011.174.
26. Kokate C K Practical Pharmacognosy 3rd edition, 1991, P.no.107-121.
27. Vermooten V (1942) The origin and development in the renal papilla of Randall's calcium plaque. J Urol 48:27–37Google Scholar
28. Kuo RL, Lingeman JE, Evan AP, Paterson RF, Parks JH, Bledsoe SB, Munch LC, Coe FL (2003) Urine calcium and volume predict coverage of renal papilla by Randall's plaque. Kidney Int 64:2150–2154CrossRefPubMedGoogle Scholar
29. Kim SC, Coe FL, Tinmouth WW, Kuo RL, Paterson RF, Parks JH, Munch LC, Evan AP, Lingeman JE (2005) Stone formation is proportional to papillary surface coverage by Randall's plaque. J Urol 173:117–119PubMedGoogle Scholar
30. Verkoelen CF, Verhulst A. Proposed mechanisms in renal tubular crystal retention. Kidney Int. 2007;72:13–18. doi: 10.1038/sj.ki.5002272. [PubMed] [Cross Ref]
31. Vermeulen CW, Lyon ES. Mechanisms of genesis and growth of calculi. Am J Med. 1968;45:684–692. doi: 10.1016/0002-9343(68)90204-0. [PubMed] [Cross Ref]

32. Finlayson B, Reid F. The expectation of free and fixed particles in urinary stone disease. Invest Urol. 1978;15:442–448. [PubMed]
33. Kok DJ, Khan SR. Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int. 1994;46:847–854. doi: 10.1038/ki.1994.341. [PubMed] [Cross Ref]
34. Escobar C, Byer KJ, Khan SR. Naturally produced crystals obtained from kidney stones are less injurious to renal tubular epithelial cells than synthetic crystals. BJU Int. 2007;100:891–897. doi: 10.1111/j.1464-410X.2007.07002.x. [PubMed] [Cross Ref]
35. Randall A. The etiology of primary renal calculus. Int Abstr Surg. 1940;71:209–240.
36. Evan A, Lingeman JE, Coe FL, Shao Y, Parks H, Bledsoe SB, Sommer AJ, Paterson RJ, Kuo RL, Kim S, Grynpas M. Crystal associated nephropathy in patients with brushite nephrolithiasis. Kidney Int. 2005;67:576–591. doi: 10.1111/j.1523-1755.2005.67114.x. [PubMed] [Cross Ref]
37. Evan AP, Lingeman J, Coe F, Shao Y, Miller N, Matlaga B, Phillips C, Sommer A, Worcester EM. Renal histopathology of stone-forming patients with distal renal tubular acidosis. Kidney Int. 2007;71:795–801. doi: 10.1038/sj.ki.5002113. [PubMed] [Cross Ref]
38. Grover PK, Marshall VR, Ryall RL. Dissolved urate salts out calcium oxalate in undiluted human urine in vitro: implications for calcium oxalate stone genesis. Chem Biol. 2003;10:271–278. [PubMed]
39. Ryall RL, Harnett RM, Marshall VR. The effect of urine, pyrophosphate, citrate, magnesium and glycosaminoglycans on the growth and aggregation of calcium oxalate crystals in vitro. Clin Chim Acta. 1981;112:349–356. [PubMed]
40. Grases F, Isern B, Sanchis P, Perello J, Torres JJ, Costa-Bauza A. Phytate acts as an inhibitor in formation of renal calculi. Front Biosci. 2007;12:2580–2587. [PubMed]
41. Lieske JC, Farell G, Deganello S. The effect of ions at the surface of calcium oxalate monohydrate crystals on cell-crystal interactions. Urol Res. 2004;32:117–123. [PubMed]
42. FARAGALLA FF, GERSHOFF SN. INTERELATIONS AMONG MAGNESIUM, VITAMIN B6, SULFUR AND PHOSPHORUS IN THE FORMATION OF KIDNEY STONES IN THE RAT. J Nutr. 1963;81:60–66. [PubMed]
43. Massey L. Magnesium therapy for nephrolithiasis. Magnes Res. 2005;18:123–126. [PubMed]

44. Worcester EM, Blumenthal SS, Beshensky AM, Lewand DL. The calcium oxalate crystal growth inhibitor protein produced by mouse kidney cortical cells in culture is osteopontin. J Bone Miner Res. 1992;7:1029–1036. [PubMed]
45. Worcester EM, Kleinman JG, Beshensky AM. Osteopontin production by cultured kidney cells. Ann NY Acad Sci. 1995;760:266–278. [PubMed]
46. Hoyer JR, Asplin JR, Otvos L., Jr Phosphorylated osteopontin peptides suppress crystallization by inhibiting the growth of calcium oxalate crystals. Kidney Int. 2001;60:77–82. [PubMed]
47. Yamate T, Kohri K, Umekawa T, Amasaki N, Isikawa Y, Kurita T. The effect of osteopontin on the adhesion of calcium oxalate crystals to Madin-Darby canine kidney cells. Eur Urol. 1996;30:388–393.[PubMed]
48. Ebrahimpour A, Perez L, Nancollas GH. Induced crystal growth of calcium oxalate monohydrate at hydroxyapatite surfaces.The influence of human serum albumin, citrate, and magnesium. Langmuir. 1991;7:577–583.
49. Dent CE, SENIOR B. Studies on the treatment of cystinuria. Br J Urol. 1955;27:317–332. [PubMed]
50. Kok DJ, Papapoulos SE, Blomen LJMJ, Bijvoet OLM. Modulation of calcium oxalate monohydrate crystallization kinetics in vitro. Kidney Int. 1988;34:346–350. [PubMed]
51. Lieske JC, Leonard R, Toback FG. Adhesion of calcium oxalate monohydrate crystals to renal epithelial cells is inhibitied by specific anions. Am J Physiol (Renal Fluid Electrolyte Physiol) 1995;268(37):F604–F612. [PubMed]
52. Wesson JA, Ganne V, Beshensky AM, Kleinman JG. Regulation by macromolecules of calcium oxalate crystal aggregation in stone formers. Urol Res. 2005;33:206–212. [PubMed]
53. www.healingrosacea.com/urinary-stonediseases/2/
54. Karadi RV, Gadge NB, Alagawadi KR, Savadi RV. Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. J Ethnopharmacol. 2006;105:306–11. [PubMed]
55. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. Kidney Int. 2003;63:1817–23. [PubMed]

56. Medeiros DM, Mustafa MA. Proximate composition, mineral content and fatty acids of cat fish (*Ictalurus punctatus* Rafinesque) for different seasons and cooking methods. J Food Sci. 1985;50:585–8.
57. Yamate T, Kohri K, Umekawa T, Iguchi M, Kurita T. Osteopontin antisense oligonucleotide inhibits adhesion of calcium oxalate crystals in Madin-Darby canine kidney cell. J Urol. 1998;160:1506–1512.[PubMed]
58. Mc Donald and Copeland. Principle of seed science and technology, 4th edition , Knurs Academic Publishers. 108,148,220.
59. Ahmed B, Alam T, Varshney M, Khan SA (2002). Hepatoprotective activity of two plants belonging to the Apiaceae and Euphorbiaceae family. J. Ethnopharmacol., 79(3): 313–316.
60. D. R. Basavaraj, C. S. Biyani, A. J. Browning, and J. J. Cartledge, "The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones," EAU-EBU Update Series, vol. 5, no. 3, pp. 126–136, 2007.
61. E. M. Worcester and F. L. Coe, "Calcium kidney stones," New England Journal ofMedicine, vol. 363, no. 10, pp. 954–963, 2010.
62. J. M. Baumann, "Stone prevention: why so little progress?" Urological Research, vol. 26, no. 2, pp. 77–81, 1998.
63. A. P. Evan, "Physiopathology and etiology of stone formation in the kidney and the urinary tract," Pediatric Nephrology, *vol. 25, no. 5, pp. 831–841, 2010.*
64. I. Sharma, W. Khan, and S. Ahmad, "In vitro and ex vivo approach for anti urolithiatic potential of bioactive fractions of gokhru with simultaneous HPLC analysis of six major metabolites and their exploration in rat plasma," Pharmaceutical Biology, vol. 55, no. 1, pp. 701–711, 2017
65. R.S. Phatak and A. S. Hendre, "In-vitro antiurolithiatic activity of Kalanchoe pinnata extract," International Journal of Pharmacognosy and Phytochemical Research, vol. 7, no. 2, pp. 275–279, 2015.
66. H. Ghelani,M. Chapala, andP. Jadav, "Diuretic andantiurolithiatic activities of an ethanolic extract of Acorus calamus L.rhizome in experimental animal models," Journal of Traditional and Complementary Medicine, vol. 6, no. 4, pp. 431–436, 2016.
67. K.Mikawlrawng and S.Kumar, "Current scenario of urolithiasis and the use of medicinal plants as antiurolithiatic agents in Manipur (North East India): a review," International Journal of Herbal Medicine, vol. 2, no. 1, pp. 1–12, 2014.

68. D. B. V. S. R. Radha, “Herbal plants used in the treatment of urolithiasis: a review,” International Journal of Pharmaceutical Research and Development, vol. 5, no. 974, pp. 66–70, 2013.
69. Ecobichon D J. The basis of Toxicity testing, 2nd Edition, CRC press, New York,1997;43-.
70. Department of Pharmacology, Toxicology & Neuroscience, Louisiana State University Health Sciences Center, Shreveport, L A,USA November 2009, Vol.47,No.9,Pages 859-869
71. 74.S. Bashir and A. H. Gilani, “Antiurolithic effect of Bergenia ligulata rhizome: an explanation of the underlying mechanisms,” Journal of Ethnopharmacology, vol. 122, no. 1, pp. 106–116, 2009
72. Rakel D. Integrative Medicine. 2nd ed. Philadelphia, Pa.:Saunders Elsevier; 2007.http://www.mdconsult.com/das/book/body/177428112-2/0/1494/0.html.AccessedJan.19,2012.
73. .Wasserstein A G.Nephrolithiasis. *American Journal of Kidney Diseases.*45;2005:422-428.
74. Monk R D, Bush insky DA.Kidney Stones.In:Kronenberg H M, Shlomo M, Polonsky
75. K S, Larsen P R,eds. *Williams Text book of Endocrinology*. 11th ed. Philadelphia, Pa: Saunders Elsevier;2008
76. Finkielstein V A. Strategies for preventing calcium oxalate stones. *CMAJ*.2006;174(10):1407-1409.
77. Straub M, Hautmann R E. Developments in stone prevention. *Curr Opin Urol*. 2005;15(2):119-126.
78. [1] H.-G. Tiselius, “Epidemiology and medical management of stone disease,” BJU International, vol. 91, no. 8, pp. 758–767,a.2003.
79. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem. 1925;66:375–81.
80. Wen C C, Nakada S Y. Treatment selection and outcomes : renal calculi. Urol Clin North Am. 2007;34(3):409-419.
81. Longo, Dan Letal (2012). *Harrison's Principals of Internal Medicine,Vol.2*.NewYork: Mc Graw-Hill.pp.2285.ISBN978-0-07-174887-2.
82. Kok DJ, Khan SR. Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int. 1994;46:847–854. [PubMed]

83. 5 *Hess B, Nakagawa Y, Coe FL. Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. Am J Physiol 1989; 257: F99 F106*
84. Pietrow P K, Preminger G M. "Evaluation and Medical Management of Urinary Lithiasis. "In: Wein A J, Kavoussi L R, Novick A C,etal. (eds.) *Wein: Campbell-WalshUrology*, 9th ed. Philadelphia, PA: Saunders;2007.
85. Miyaoka R, Monga M: Use of traditional Chinese medicine in the management of urinary stone disease. Int Braz J Urol. 2009; 35: 396-405.
86. Kieley S, Dwivedi R, Monga M: Ayurvedic medicine and renal calculi. J Endourol.2008; 22: 1613-6. F
87. *Hennequin C, Lalanne V, Daudon M, Lacour B, Drüeke T. A new approach to studying inhibitors of calcium oxalate crystal growth. Urol Res 1993; 21: 101 8*
88. Duarte J D, Cooper-De Hoff R M (June 2010). "Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide like diuretics".*Expert RevCardio vasc Ther* **8**(6): 793–802. doi:10.1586/erc.10.27.PMC 2904515. PMID20528637.
89. Finkielstein V A. Strategies for preventing calcium oxalate stones. *CMAJ* 2006;174(10):1407-1409.
90. //www.ncbi.nlm.nih.gov/pmc/articles/PMC 2904515/.
91. Environmental Health & Safety M S D S Number:P5675
92. Prasad KVSRG, Sujatha D, Bharathi K: Herbal Drugs in Urolithiasis - A Review. Phycog Rev. 2007; 1: 175-9.
93. Barros ME, Schor N, Boim MA: Effects of an aqueous extract from Phyllantus niruri on calcium oxalate crystallization in vitro. Urol Res. 2003; 30: 374-927.Vermooten V (1942) The origin and development in the renal papilla of Randall's calcium plaque. J Urol 48:27–37Google Scholar
94. Wang AY. Vascular and other tissue calcification in peritoneal dialysis patients. Perit Dial Int. 2009;29(Suppl 2):S9–S14. [PubMed]
95. Khan SR. Animal models of kidney stone formation: an analysis. World J Urol. 1997;15:236–243.[PubMed]
96. Kato Y, Yamaguchi S, Yachiku S, Nakazono S, Hori J, Wada N, Hou K. Changes in urinary parameters after oral administration of potassium-sodium citrate and magnesium oxide to prevent urolithiasis. Urology. 2004;63:7–11. discussion 11–12. [PubMed]

97. Atmani F, Khan SR. Effects of an extract from Herniaria hirsuta on calcium oxalate crystallization in vitro. BJU Int. 2000;85:621–625. [PubMed]
98. Aihara K, Byer KJ, Khan SR. Calcium phosphate-induced renal epithelial injury and stone formation: involvement of reactive oxygen species. Kidney Int. 2003;64:1283–1291. [PubMed]
99. Byer K, Khan SR. Citrate provides protection against oxalate and calcium oxalate crystal induced oxidative damage to renal epithelium. J Urol. 2005;173:640–646. [PubMed]
100. Santhosh Kumar M, Selvam R. Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. The Journal of Nutritional Biochemistry. 2003;14:306–313. [PubMed]
101. 104. Wiessner JH, Hasegawa AT, Hung LY, Mandel GS, Mandel NS. Mechanisms of calcium oxalate crystal attachment to injured renal collecting duct cells. Kidney Int. 2001;59:637–644. [PubMed]
102. 105.http://www3.interscience.wiley.com/journal/112603635/abstract?CRETRY=1&SRETRY=0

ANNEXURE

INSTITUTE OF HERBAL SCIENCE
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AUTHENTICATION CERTIFICATE

Based upon the Organoleptic /macroscopic /~~microscopic~~ examination of fresh /market sample, it is certified that the specimen given by S.Sumalatha, M.Pharm (Dept. of pharmacology), C.L. Baid Metha college of pharmacy. is identified as below:

Binomial: Apium graveolens L.

Family: Umbelliferae

Synonym(s): -

Regional names: English : celery

Reg.No of the certificate: PARC/2017/3460

References: Nair, N.C & Henry, A.N. Flora of TamilNadu, India I: Pg : 178 .1983.

Henry, A.N. *et al.* Ibid. ——— II: ——— .1987.

Ibid. ——— III: ——— .1989.

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